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PANCREATIC SECRETIONS IN THE GROWING PIG

by

JOHN H. HEE



A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE
OF MASTER OF SCIENCE

IN

ANIMAL NUTRITION

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I would like to dedicate this thesis to Dave Kuzyk and
to the memory of the late Nick Rudski.

...one flew east, one flew west...

ABSTRACT

Ten Yorkshire X Lacombe barrows (30 kg average liveweight) were successfully prepared for collection of pancreatic juice with draining and re-entrant cannulas. The procedure involved the construction of a duodenal pouch at the pancreatic duct and end-to-end anastomosis of the duodenum. Success with this procedure is attributed to fistula location, one-way valve design of the re-entrant arm of the cannula and precautions taken to minimize the formation of intra-abdominal adhesions.

Three experiments were conducted at the University of Alberta Metabolic Barn utilizing the ten surgically altered pigs to determine the effect of diet and feeding on pancreatic secretions. In the first two experiments, pancreatic juice was collected between meals from 6 pigs fed partially-purified diets in 2 meals per day. In Experiment 1 (3 pigs), dietary treatment had no effect ($P > 0.05$) on volume or protein content. Lipase activity increased 6-fold ($P < 0.05$) with the feeding of 10% tallow as compared to 2% tallow included in the diets. Trypsin, chymotrypsin and amylase activities decreased 2-fold ($P < 0.05$) with the absence of dietary protein. In Experiment 2 (3 pigs), dietary treatment had no effect ($P > 0.05$) on volume. All four enzyme activities and protein content decreased with the absence of dietary protein ($P < 0.05$). It was concluded that enzyme composition changes in response to diet.

In a third experiment, pancreatic juice was collected for 24 h collection periods from 4 pigs fed a grower diet in one meal, two meals or three meals per day. A trend ($P < 0.05$) for increased volume, protein and all four enzyme activities after feeding was observed. Daily volume tended to increase ($P < 0.01$) by 0.5 l with each additional meal. Frequency of feeding had no effect ($P > 0.01$) on protein secretion and proteolytic enzyme activities. Amylase activity increased 2-fold ($P < 0.01$) with each additional meal. Lipase activity was higher ($P < 0.01$) when pigs were fed one meal per day as compared to two meals per day. Feeding stimulated pancreatic secretion and frequency of feeding increased daily volume and amylase activity.

Key Words: CANNULATION, DIET, FEEDING, PANCREATIC ENZYMES, SWINE

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INTRODUCTION *sl*

The pancreas secretes a clear, viscous solution with an osmolarity similar to that of serum into the proximal duodenum, where it is mixed with intestinal contents. This solution consists of two fractions. The aqueous fraction buffers stomach acid so that the enzyme fraction can promote digestion.

Water and electrolytes are secreted from the pancreas by the epithelial cells of the intercalated ducts. The four predominant electrolytes are sodium, potassium, bicarbonate and chloride. The bicarbonate concentration of pancreatic secretions can be three to four times greater than that in serum and has been found to increase with the rate of pancreatic secretion while chloride concentration decreases to maintain a constant osmolarity. The bicarbonate-chloride exchange is an active process within the ducts. The high concentration of bicarbonate ion helps to neutralize the acid chyme so that digestive enzymes can act at their optimal pH.

The digestive enzymes of the pancreas are capable of facilitating the hydrolysis of proteins, carbohydrates and fats to simple molecules that can be readily absorbed by the small intestine. The enzymes are stored as inactive precursors in zymogen granules in the apex of the acinar cells within the pancreas.

The major proteolytic enzymes are trypsin, chymotrypsin and carboxypolypeptidase. They are synthesized and secreted

in their inactive forms as proenzymes. Trypsinogen, the trypsin proenzyme, becomes activated upon secretion into the duodenum by enterokinase, an enzyme produced by the intestinal mucosa. Trypsin is autocatalytic and activates trypsinogen. Trypsin also activates the remaining proenzymes. The proteolytic enzymes are responsible for the hydrolysis of whole and partially digested proteins into small peptides and free amino acids.

The digestive enzyme that hydrolyzes carbohydrates such as starch and glycogen to form disaccharides is α -amylase. It is activated by chloride ions.

Lipase hydrolyzes triacylglycerols releasing long chain fatty acids. It hydrolyzes most fat to form 2-monoacylglycerols, as well as, diacylglycerols, glycerol and free fatty acids. Colipase prevents bile salt inactivation of lipase.

Thus, the digestive enzymes of the pancreas play a major role in nutrient absorption. They contribute to the breakdown of diverse, complex molecules of dietary components into simple molecules suitable for absorption.

The study of the exocrine pancreas has developed from *in vitro* methods using slaughter techniques to successful, longterm *in vivo* measurements to quantitate pancreatic secretions. Cannulation has given access to pancreatic secretions with minimal interference to normal digestive function.

The major objectives of this work were to develop a method to collect pancreatic juice from the growing pig and to investigate the enzyme fraction of pancreatic secretions in response to diet and feeding.

I. RE-ENTRANT DIVERSION OF PANCREATIC SECRETIONS

A. Abstract

A surgical procedure was developed to permit collection of pancreatic juice from conscious, unrestrained pigs. A cannula was used to conduct pancreatic juice from a small isolated segment of duodenum at the pancreatic duct, through permanent intercostal fistulas, and back into the duodenum within 3 cm of the normal entry point for pancreatic juice. Success with this procedure is attributed to fistula location, one-way valve design of the re-entrant arm of the cannula and precautions taken to minimize the formation of intra-abdominal adhesions. Pancreatic juice was collected from 3 barrows (35 kg average initial weight) which were fed cornstarch-based diets twice daily, 0.9 kg each meal, 12 h apart for a 6 week period. The average daily volume of pancreatic juice secreted was 3.8 ± 0.9 l which contained 14.4 ± 3.5 g protein. A 12 h secretion profile related to meal time, showed a consistently repeated response pattern to feeding. Secretion rates increased within the first 3 h, peaked during 3-7 h ($P < 0.05$) and decreased to basal rates during the last 5 h. Secretions obtained from blind 5 cm duodenal pouches did not exceed 20 ml per day. During the four month study, pancreatic secretions were persistent and meal induced secretion patterns were sustained. Cannulas were still functional when pigs achieved liveweights greater than 100 kg.

Key Words: CANNULATION, PANCREAS, SWINE

B. Introduction

Studies on the influence of diet on the exocrine pancreatic secretion of growing pigs have been hampered by the premature loss of pancreatic duct catheter patency (Partridge *et al.* 1982). Such practical problems have precluded long term studies for assessing the relative role and adaptive capability of the exocrine pancreas in digestion. An alternative was sought for chronic cannulation of the pancreatic duct in order to overcome the difficulties of interpreting results obtained in short-term studies.

Two basic approaches have been used to collect pancreatic secretions in experimental animals: (1) direct cannulation of the pancreatic duct and (2) collection of pancreatic juice from a duodenal pouch. Thomas (1959) reviewed the use of these two approaches in dogs. Direct cannulation procedures have been described for rats (Love 1957), sheep (Taylor 1960), swine (Pekas 1965), cattle (Wass 1965a) and poultry (Hulan *et al.* 1972). However, this approach was found to be of limited success in growing pigs (Corring *et al.* 1972) as pancreatic juice could be collected for only 1 or 2 months following surgery. Corring (1980) and co-workers adopted this approach on the belief that the trauma of direct duct cannulation was not as great as that caused by duodenal pouch formation and intestinal anastomosis.

Recently, Zebrowska *et al.* (1981) attempted an intermediate approach by preparing a duodenal pouch and connecting the remaining ends of the duodenum with a re-entrant cannula. Unfortunately, these workers did not reconstruct the duodenum and may have compromised pancreatic function by possibly altering the rate of passage through their re-entrant cannula.

A procedure was devised that reduced problems previously associated with cannulation to collect pancreatic juice in growing pigs.

C. Materials and Methods

Cannula design

Cannulas were made from preformed Silastic® components (Dow Corning Corp., Midland, Michigan) and assembled with RTV silicone cement (Figure 1.1). Polyvinyl alcohol foam (Ivalon® sponge) was purchased from Unipoint Industries Inc., High Point, N.C.

Animals

Three Yorkshire X Lacombe barrows (28 kg average liveweight) were obtained from the University of Alberta swine herd. Pigs were housed individually in 0.5 x 1.0 m stainless steel metabolism crates in an air conditioned and light controlled barn (continuous light, air temperature 23 ± 1 °C) one week prior to surgery and fed an 18% crude

protein pelleted starter diet (Table I.1) *ad libitum*. Water was supplied *ad libitum* from a low pressure drinking nipple.

Surgical procedures

Pigs (30 kg average liveweight) were starved for 36 h and anesthetized with halothane-oxygen through a face mask. No premedication was used. Aseptic technique and standard surgical procedures were followed.

The pig was placed in left lateral recumbency, the right thoracic and abdominal wall was clipped with a #40 blade, scrubbed with providone iodine solution, rinsed with 70% ethanol and draped.

A 15 cm incision was made caudad and parallel to the last rib through the skin and the external abdominal oblique muscle. The remaining two muscle layers were split by blunt dissection and the peritoneum was cut parallel to the incision. Thereafter, 250 ml of sterile saline were poured into the peritoneal cavity.

The right side of the ribcage was elevated and the proximal duodenum was retracted with intestinal forceps. Saline moistened Telfa® pads, 10 cm², (Kendall Canada, Toronto, Ontario) were placed over the wound edges beneath the exposed section of duodenum. The pancreatic duct, located approximately 15 cm from the pyloric sphincter where the pancreas is most closely attached to the duodenum, was identified within pancreatic tissue by gentle palpation.

A 4-6 cm section of duodenum which received the pancreatic duct was isolated to prepare a pouch. The duodenum was clamped tightly 2-3 cm caudal to the pancreatic duct with two 18.5 cm nontraumatic Bainbridge vascular clamps (Figure I.2, A) and transected. If major vessels could not be avoided, these were ligated with 3-0 silk before the duodenum was transected between the clamps. The proximal end of the duodenum, which was closed with 2-0 chromic gut by a Parker-Kerr oversew, formed the bottom of the duodenal pouch.

The intestinal contents of the pouch that was being prepared were evacuated by gentle, retrograde massaging. A pair of clamps was placed 2-3 cm anterior to the duct (Figure I.2, B) and the duodenum was transected between the clamps. A single purse string (2-0 chromic gut) was placed around the opening of the pouch and the draining arm of the cannula was inserted and secured. As the draining arm of the cannula filled with pancreatic juice, care was taken to divert the secretions away from the abdominal cavity.

The completed pouch was gently reflected dorsally and the remaining duodenum was anastomosed with one layer of interrupted inverting mattress sutures of 3-0 gut at 3 mm intervals (Figures I.3, I.4). A 2 cm oval purse string (2-0 chromic gut) was placed 1 cm orad to the anastomosis on the antemesenteric side to secure the re-entrant arm of the cannula. The pouch and draining arm of the cannula were rinsed of small blood clots and mucus by passing a small

catheter through the cannula and flushing with saline. The cannula was exteriorized through stab wounds between the last 3 ribs 15 cm dorsal to the costochondral junction with the re-entrant arm of the cannula positioned caudally. The pouch and the anastomosed duodenum (Figure 1.5) were held against the peritoneum by the cannula which was secured against the skin with the rigid washers and retaining rings (Figure 1.1, #3,4). Before closing the abdominal cavity, 200 mg oxytetracycline hydrochloride and an additional 250 ml of sterile saline were introduced into the peritoneal cavity. Thus, a total of 0.5 l of saline was delivered in an attempt to maintain the animal's fluid balance.

Following surgery, the pigs were allowed to recover in raised pens with plastic coated wiremesh flooring and a heat lamp placed directly overhead. Water and the starter diet were supplied *ad libitum*. The next day pigs were moved to metabolism crates in the same barn and were offered the starter diet *ad libitum* for 2 weeks to attain a liveweight of 35 kg. Thereafter, they were fed cornstarch-based diets (Table 1.2), 900 g twice daily, at 0800 and 2000 h for 6 weeks. Water was supplied *ad libitum*.

Collection of pancreatic juice

Collection of pancreatic juice involved disconnection of the re-entrant cannula and attachment of extension tubing (3.2 mm id x 2 m). Straight stainless steel tubing (5 cm) was used to attach the cannula to the extension tubing. To

prevent entanglement and to enable unobstructed continuous collection, the extension tubing was taped to the base of the tail. Pancreatic juice was reintroduced by gravity flow every hour to prevent dehydration due to the loss of electrolytes via pancreatic secretions (Thomas 1959) and to minimize hypersecretion (Corring 1974). Collections were made every 2 weeks on days 9 (0800h to 2000h) and 13 (2000h to 0800h) from three pigs over a 6 week period.

D. Results and Discussion

The entire surgical procedure was completed within 1 h and pigs usually regained consciousness minutes after discontinuing anaesthesia. Recovery from surgery was rapid and uneventful. Pigs started consuming the starter diet and drinking water within 12 h after surgery and all pigs consumed more than 1.5 kg diet per day within the week following surgery. Pigs usually passed hard dry feces within the week as they began to increase their consumption of the starter diet.

Post-surgical complications were encountered in the initial cannulations. These were characterized by inappetance and vomiting which were the consequence of intestinal tract blockage secondary to intra-abdominal adhesion formation. These complications required repeated surgery, but carried a poor prognosis. Formation of adhesions were prevented by modifying the surgical procedure to minimize abrasion and contact with rough surfaces.

The duodenum and pancreas can be adequately exposed through the approach that was previously described. However, considerable variability in the length of the mesoduodenum was found which at times seriously hampered the surgical procedure. Manipulation of the intestines was made easier by pouring sterile saline (at room temperature) into the peritoneal cavity at the beginning of surgery causing smooth muscle contraction and increased tone of the gut. The smooth contact surface provided by the Telfa® pads (or adhesive drapes) further reduced tissue damage. Surgical gloves with a textured finish should be avoided.

Hemostasis was maintained by ligating major blood vessels and by using vascular clamps when transecting the duodenum. The narrow clamps crushed the blood vessels, prevented slippage of the clamped intestine and reduced tissue loss.

During preparation of the anastomosis (Figure 1.4), contact between the viscera and the crushed ends of the duodenum was avoided and suture knots were hidden between the serosal layers of the two ends of duodenum by using interrupted inverting mattress sutures. This procedure prevented formation of adhesions between the anastomosed duodenum and adjacent viscera. The use of three stay sutures provided for easier manipulation of the duodenum when the clamps were removed to finish the anastomosis. By tenting the duodenum with the middle and opposing stay sutures, the two edges of the duodenum that remained to be anastomosed

were brought into close opposition. This prevented intestinal contents from leaking out into the abdominal cavity.

The final instillation of saline into the abdominal cavity probably helped to re-establish the normal alignment of the viscera once the animals regained consciousness and stood on their feet. Following anaesthesia, all pigs were ambulatory within 15 min after surgery. The use of longer acting barbituate anaesthesia could lead to a longer period of recumbency and might promote the formation of intestinal adhesions and subsequent complications.

Post-mortem examinations were carried out 4 months later when the pigs achieved liveweights greater than 100 kg and became too large for the metabolic crates. No evidence of intestinal adhesions was found, but there were excellent adhesions between the parietal peritoneum, the pouch, and the anastomosed duodenum. There was no dilatation of the pouch and the mucosa showed no evidence of irritation or damage.

In earlier preparations, the pouch was sutured to the anastomosed duodenum to prevent twisting and stretching of the vascular attachments. However, at times a fistula formed allowing pancreatic juice to flow directly into the duodenum. Adhesions between intestinal loops will fistulate and can permanently alter the direction of intestinal flow. Fistula formation is not uncommon at adhesion sites and may explain inconsistent results in some studies involving the

use of gastro-intestinal cannulas. Similar complications have been observed in sheep fitted with intestinal cannulas at the University of Alberta (R. J. Christopherson, personal communication). Post-mortem examination of surgically altered animals should be carried out routinely.

Considerable variability has been reported in pancreatic duct anatomy in pigs. Embryologically, the pancreas is derived from two separate primordia. One primordium arises dorsally, directly from the duodenal entoderm; the other arises ventrally, from the hepatic diverticulum. These two primordia eventually fuse to form the pancreas. In the pig, the terminal portion of the pancreatic duct associated with the common bile duct atrophies to become a remnant leaving the accessory pancreatic duct as the definitive duct draining the pancreas (Patten, 1948). Vodovar *et al.* (1964) observed that in 50 Large White pigs, 9 had both ducts, 2 had drainage into the common bile duct and 39 had a single duct joining the duodenum. Wass (1965b) examined the pancreatic duct system in 15 pigs of mixed breeds using an x-ray technique following injection of a radiopaque contrast medium into the pancreatic duct. Only the normal pancreatic duct was functional in each pig and no abnormalities were found. Examination of 30 pigs from the University of Alberta swine herd showed that the pancreatic duct was entirely embedded in pancreatic tissue and could be discerned through gentle palpation. No duct abnormalities were found upon dissection.

The cannula used in the present studies incorporates several important design features. The retaining rings and washers are necessary to immobilize the cannula and hold the duodenum tightly against the body wall to prevent twisting of the intestine. The polyvinyl alcohol foam reduces stretching of intercostal fistulas and subsequent leakage which can cause irritation and rubbing due to an alkaline dermatitis as was described by Scott (1940). Without the rigid internal washers and polyvinyl alcohol foam, the pigs would rub against the plexiglass wall of the metabolism crate and eventually dislodge the cannula. In further studies, the adhesive bond between the polyvinyl chloride washers and the silicone rubber tubing was found to be unreliable. Pigs that made a habit of rubbing against the plexiglass wall of the metabolism crate broke the bond between the washers and tubing of the cannula. A further improvement of the cannula can be made by adapting the design to a one piece cannula which would eliminate the bonding problem.

A one-way valve in the cannula is important. Pekas (1965) found in pigs with re-entrant cannulae that during intervals of high peristaltic pressures in the duodenum the flow of secretions reversed and ingesta passed into the pancreas. A 30% mortality occurred when bile was injected into the pancreas via the pancreatic duct (Landy, 1966). The thin silicone rubber valve at the re-entrant port of the cannula prevented backflow of digesta, but created little

resistance to flow (< 6 mm pancreatic juice).

The perforated basket on the draining port of the cannula ensured uninterrupted flow without damaging the mucosa. The dimensions of the silicone rubber tubing were adequate to prevent occlusion yet sufficient to avoid irritation. The flow of pancreatic juice was continuous even when pigs lay directly on the tubing.

Pancreatic secretions were not interrupted by surgery. From one pig, 800 ml were collected between 16 and 22 h after surgery. This amount compared very favorably to subsequent collections. The secretions were clear, flow was a continuous drip and the average pH was 8.4. Volume and protein content were consistent from day to day. The rate of secretion of pancreatic juice following each feeding showed a characteristic profile (Figure 1.6). Post-prandial flow rates increased within the first 3 h. Maximum volumes were secreted within 3-7 h and were followed by a decline during the last 5 h. Secretions during 2-7 h were significantly greater than during 8-12 h ($P < 0.05$). The average daily collection was $3.8 \pm 0.9(18)$ l (mean $\pm$ SD) which contained $14.4 \pm 3.5(18)$ g (mean $\pm$ SD) protein. Dietary treatment had no effect ($P > 0.05$) on daily volume or protein content. To measure the secretory contribution by the duodenal pouch, two pigs were prepared using the same surgical procedure, but the pouches were made from portions of the duodenum on either side of the pancreatic duct. Flow from the blind pouches appeared to be constant, but volumes did not exceed

10 ml over 12 h. Hence, less than 0.5% of daily collections were of extra pancreatic origin.

Considerable variation has been reported in the literature for daily volume and protein content of pancreatic juice (Table 1.3). Values observed in the present studies for three pigs during their growth from 35 to 50 kg fall within the range of values reported in the literature. Although a comparison of these reports is confounded by feed intake, diet composition, pig weight and subsequent growth rates, the collection of pancreatic juice from a duodenal pouch is a promising alternative to direct duct cannulation.

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TABLE 1.1. Formulation and partial chemical composition of the starter diet.

Ingredients(% as fed):

Wheat	25.0
Barley	25.0
Oat groats	25.0
Soybean meal	18.0
Tallow	3.0
Calcium phosphate	1.0
Calcium carbonate	1.0
Iodized salt	0.5
Vitamin-mineral premix ¹	1.0

Chemical analyses:

Dry Matter(%)	86.5
Gross Energy(MJ/kg)	16.7
Ether Extract(%)	5.6
Crude Protein(%)	17.5
Ash(%)	4.3

The premix provided the following per kg of diet:
(1) minerals - 120 mg zinc, 48 mg manganese, 100 mg iron, 10 mg copper, .1 mg selenium;
(2) vitamins - 7500 IU vitamin A, 700 IU vitamin D₃, 45 IU vitamin E, 12 mg riboflavin, 40 mg niacin, 25 mg pantothenic acid, 28 µg B₁₂.

TABLE 1.2. Formulation and partial chemical composition of the high fat, control and protein-free diets.

Diets:	High Fat	Control	Protein Free
<i>Ingredients(% as fed):</i>			
Cornstarch	38.05	46.05	78.70
Soybean meal	33.30	33.30	-
Dextrose	10.00	10.00	10.00
Cellulose ¹	5.00	5.00	5.00
Tallow	10.00	2.00	2.00
Calcium phosphate	1.40	1.40	2.40
Calcium carbonate	0.70	0.70	0.40
Trace mineralized salt ²	0.50	0.50	0.50
Vitamin-mineral premix ³	1.00	1.00	1.00
Methionine	0.05	0.05	-
<i>Chemical analyses:</i>			
Dry Matter(%)	90.51	90.10	90.64
Gross Energy(MJ/kg)	17.66	15.69	14.90
Ether Extract(%)	9.74	1.52	1.50
Crude Protein(%)	14.80	14.47	0.30
Ash(%)	4.97	4.54	3.30

¹ Alphafloc®, Brown Company, Berlin, N.H.

² Supplied by Windsor Salt Co., Toronto, Ontario.
Composition(percentage): 96.5 NaCl, .40 ZnO,
.16 FeCO₃, .12 MnO, .033 CuO, .007 Ca(IO₃)₂,
.004 CoO.

³ The premix provided the following per kg of diet:
(1) minerals - 100 mg zinc, 20 mg manganese, 150 mg iron, 10 mg copper, .1 mg selenium;
(2) vitamins - 1300 IU vitamin A, 150 IU vitamin D₃, 11 IU vitamin E, 2 mg menadione, .1 mg biotin, .6 mg folic acid, 12 mg niacin, 11 mg pantothenic acid, 1.1 mg pyridoxine, 2.2 mg riboflavin, 1.1 mg thiamine, 11 µg B₁₂, 55 mg choline chloride.

TABLE I.3. Reported values for daily volume and protein content of pancreatic juice collected from growing pigs.

AUTHORS	FEED INTAKE (kg. diet)	VOLUME (l)	PROTEIN (g)	LIVEWEIGHT (kg)	SURGICAL PROCEDURE
Corring <i>et al.</i> (1972)	0.9, barley-soya	1.75	6.1	42	duct catheter
Corring (1980)	1.0, barley-soya	2.5	18.6	45	duct catheter
Partridge <i>et al.</i> (1982)	1.6, barley-fishmeal 1.6, starch-casein	5.0 1.3	9.8 6.7	48 48	duct catheter
Zebrowska <i>et al.</i> (1981)	1.5, barley-soya 1.5, starch-casein	2.2 1.2	12.1 10.9	40 40	pouch and duodenal re-entrant
Present study	1.8, starch-soya high fat control protein-free	3.8 3.9 4.0 3.5	14.4 15.3 15.0 12.2	35-50	pouch and anastomosis

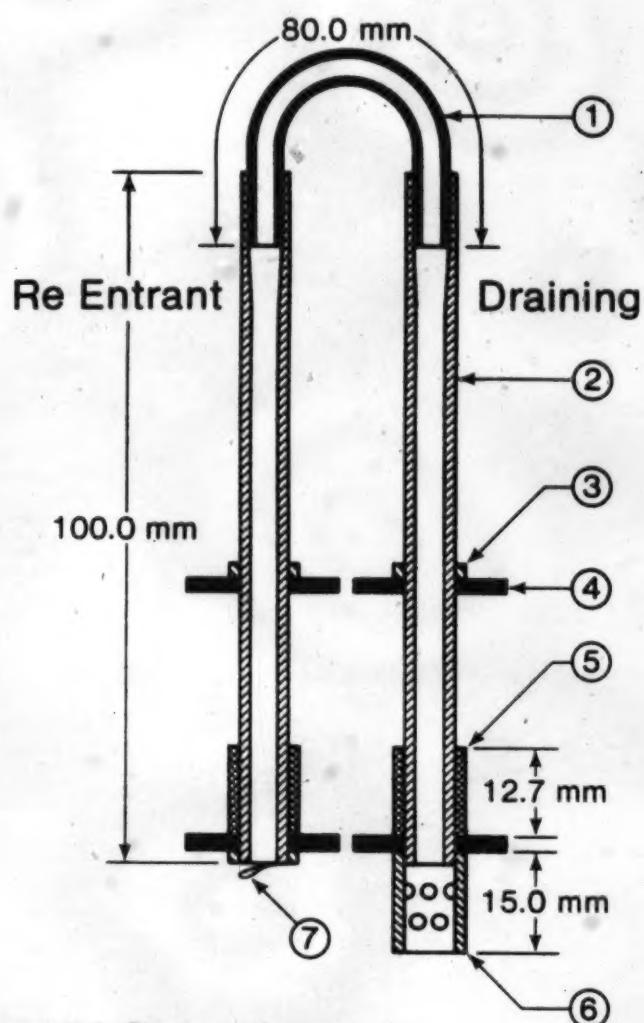


Figure I.1: Pancreatic cannula

- (1) connector
(4.8 mm × 2.8 mm #304 stainless steel tubing)
- (2) draining and re-entrant arms of cannula
(3.4 mm × 6.4 mm silicone rubber tubing)
- (3) retaining rings
(6.4 mm × 9.5 mm silicone rubber tubing)
- (4) rigid washers
(2.0 mm polyvinyl chloride)
- (5) collars
(2.0 mm polyvinyl alcohol foam)
- (6) basket
(6.4 mm × 9.5 mm silicone rubber tubing)
- (7) one-way valve
(0.2 mm silicone rubber sheeting)

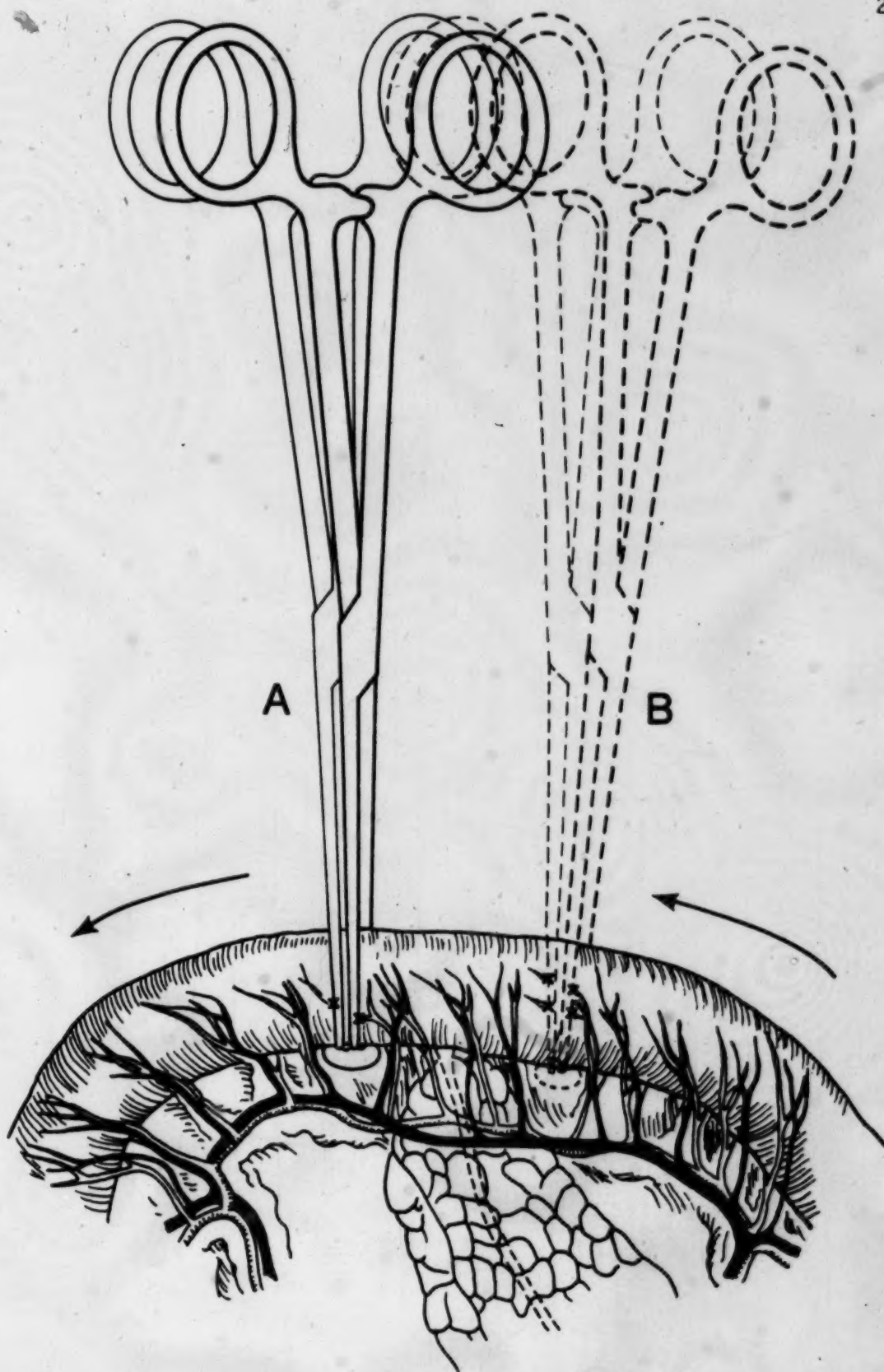


Figure I. 2. Placement of intestinal clamps

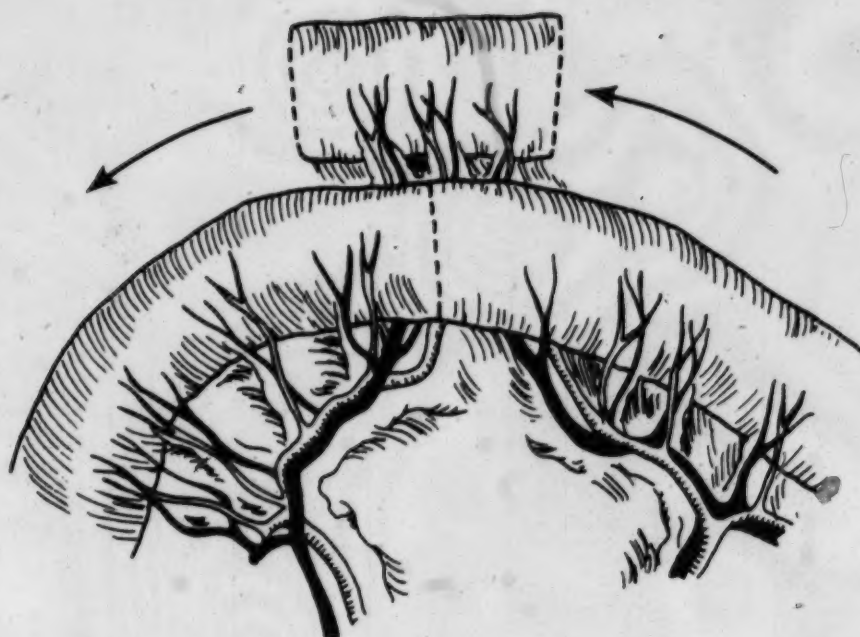


Figure I. 3. Anastomosis and duodenal pouch

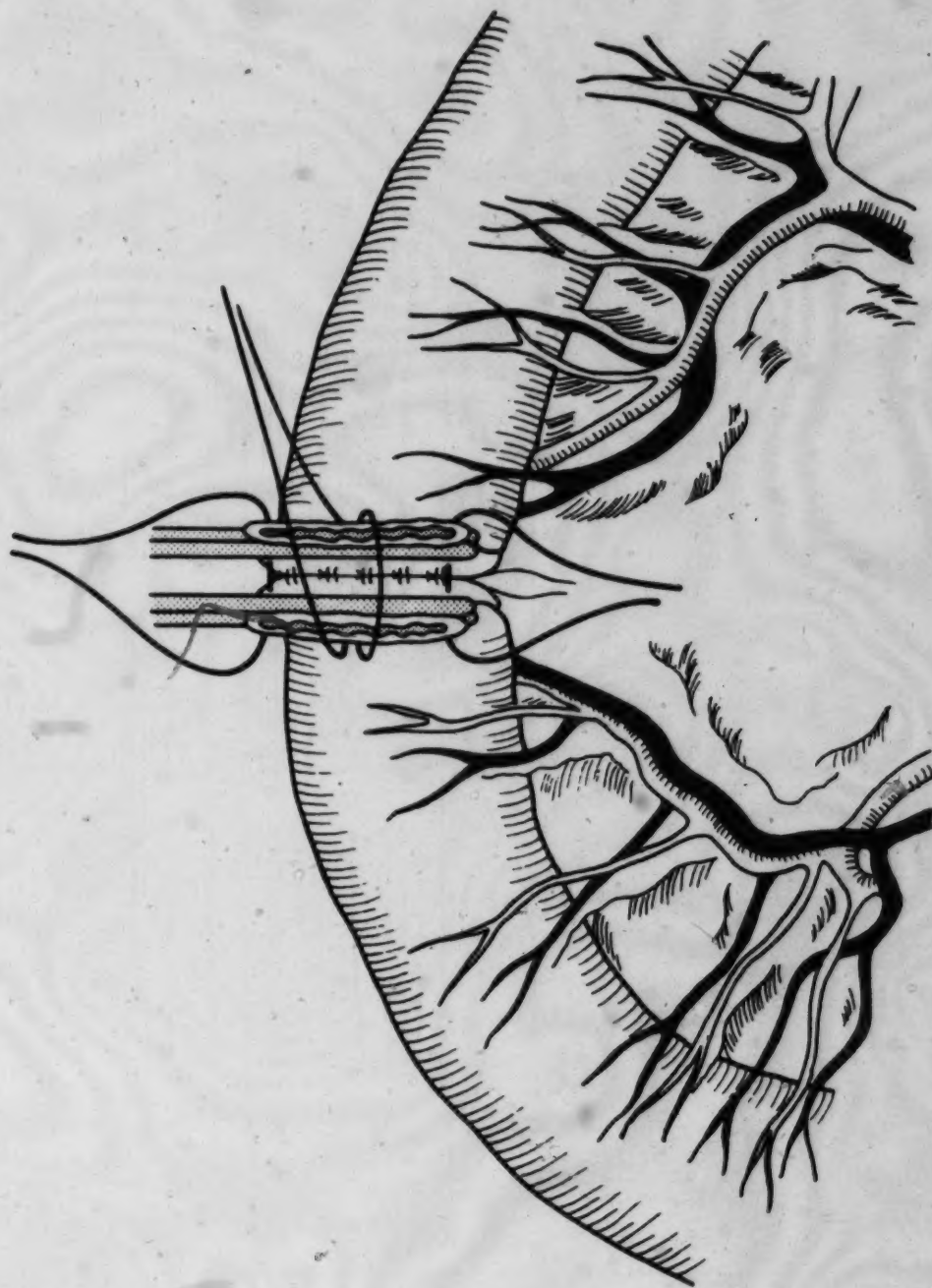


Figure I. 4. End-to-end anastomosis

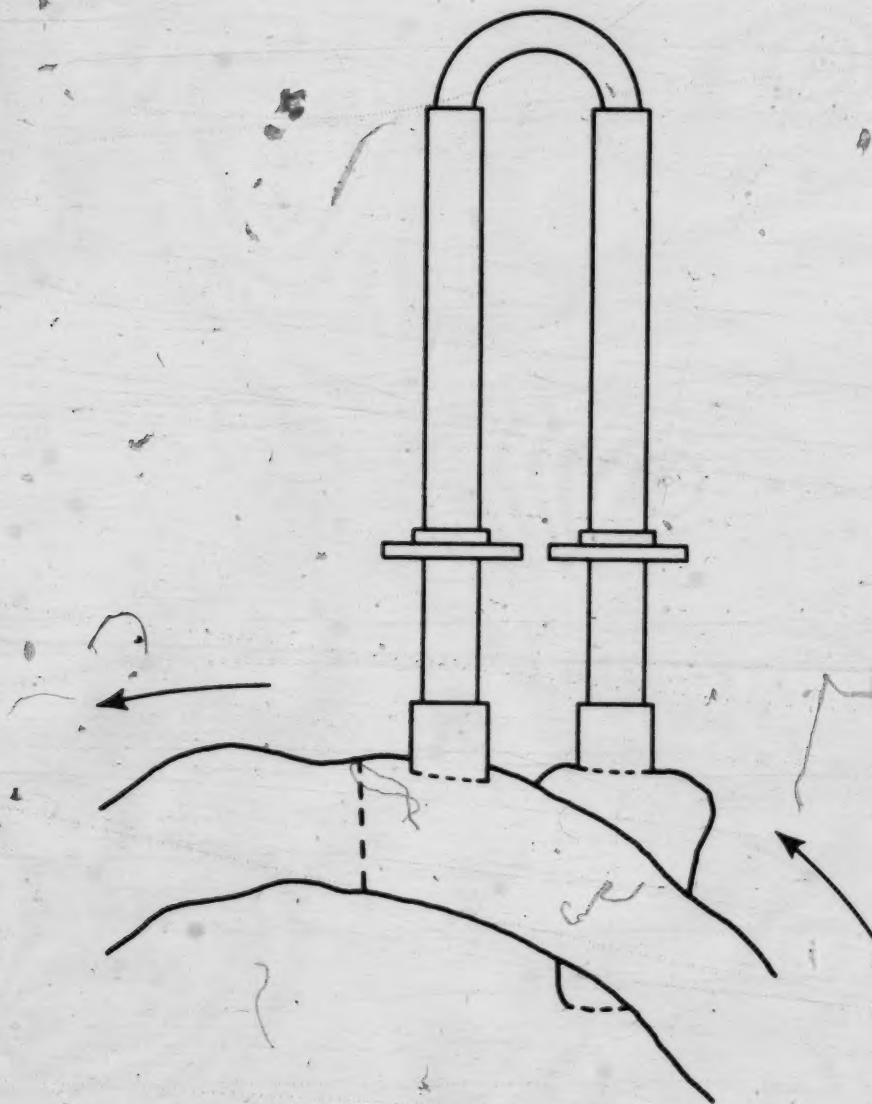


Figure 1.5. Placement of pancreatic cannula

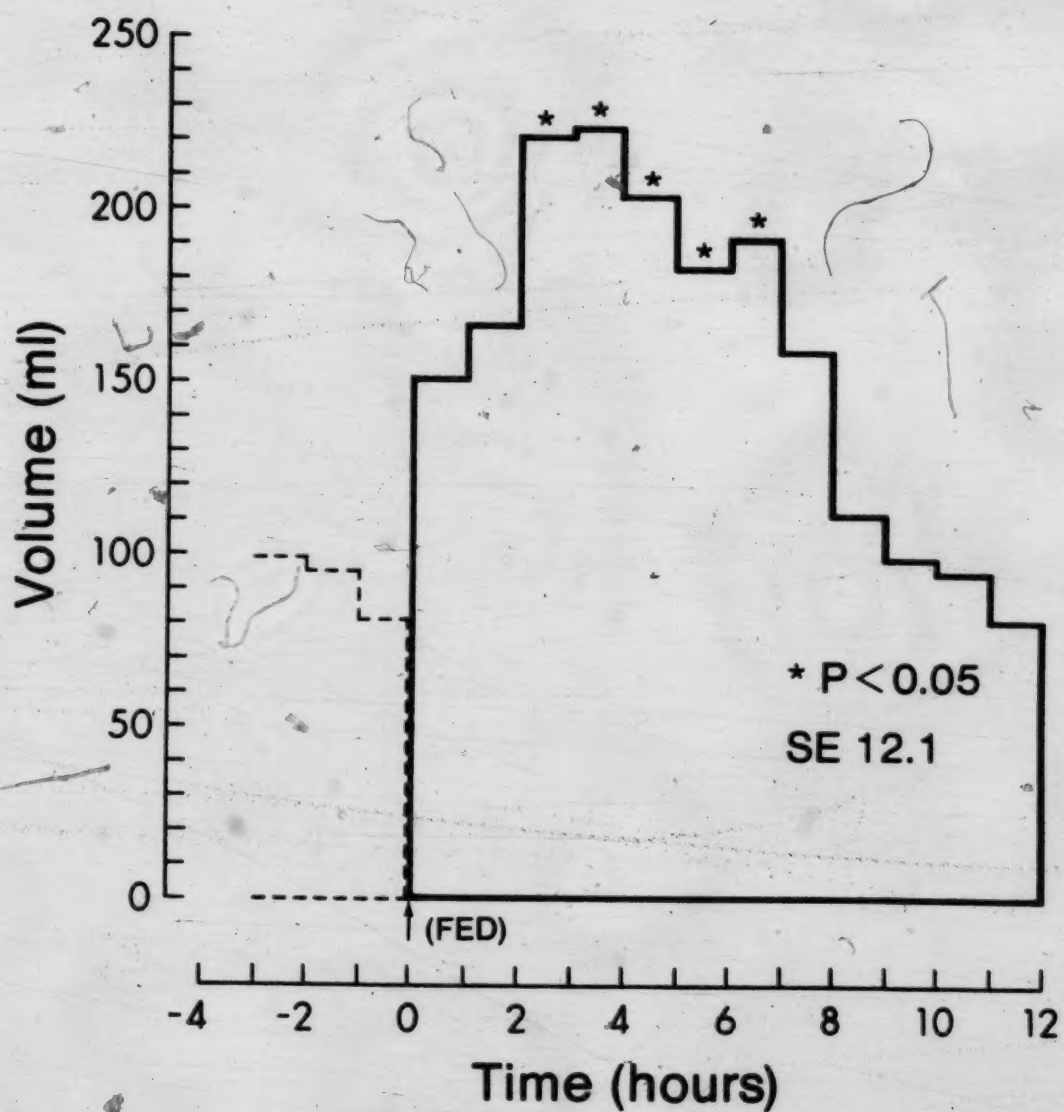


Figure I.6. Pancreatic secretion following a meal

II. EFFECT OF DIET ON PANCREATIC SECRETIONS

A. Abstract

Two experiments were conducted to determine the relationship between diet composition and pancreatic exocrine secretion in pigs that were surgically prepared for collection of pancreatic juice. In the first experiment, 3 barrows (35 kg average initial weight) were fitted with pancreatic cannulas and used in a randomized complete block design. The three pigs were fed one of three cornstarch-based diets (HF, high fat - 14% crude protein, 10% tallow; C, control - 14% crude protein, 2% tallow; PF, protein-free - 0% crude protein, 2% tallow) at 0800h and 2000h, 0.9 kg each meal, for a 14-day period for each diet. Pancreatic juice was collected on days 9 (0800h to 2000h) and 13 (2000h to 0800h) of each test period. Dietary treatment had no effect ($P > 0.05$) on volume or protein content. Lipase activity increased 6-fold ($P < 0.05$) with the feeding of 10% tallow as compared to 2% tallow included in the diets. Trypsin, chymotrypsin and amylase activities decreased 2-fold ($P < 0.05$) with the absence of dietary protein. In the second experiment, the control and protein-free treatments were repeated with another 3 barrows (35 kg average initial weight) fitted with pancreatic cannulas. Dietary treatment had no effect ($P > 0.05$) on volume. All four enzyme activities and protein content decreased with the absence of dietary protein ($P < 0.05$). It

was concluded that enzyme composition changes in response to diet.

Key Words: CANNULATION, ENZYMES, PANCREAS, SWINE

B. Introduction

Pancreatic secretions contribute to the hydrolysis of dietary proteins, carbohydrates and lipids. The principal pancreatic enzymes (trypsin, chymotrypsin, amylase and lipase) break these major constituents of the diet down to smaller molecules that can be readily absorbed by the small intestine. Deficiencies in pancreatic enzymes or intestinal enterokinase can lead to malnutrition and growth failure (Haworth *et al.* 1971, Tarlow *et al.* 1970). In pigs, the apparent digestibility of nitrogen and energy decreased by 12.9% and 3.1% respectively, 5 days following diversion of pancreatic juice (Corring and Bourdon 1976). Corring and Bourdon (1977) found a larger reduction in apparent digestibility of nitrogen and energy, namely 35.6% and 12.1% respectively, within 25 days after ligation of the pancreatic duct. They reported a compensation 87 days after pancreatic duct ligation, but the origin of the compensation was unknown and may have been due to the re-establishment of the duct with the duodenum. The previous studies point out the importance of pancreatic enzymes in digestion.

Pavlov (1910) proposed that the enzyme composition of pancreatic secretions depends on the dietary components of the ingested meal. This concept of digestive enzyme

adaptation to the diet was questioned by Babkin (1950) who stated that pancreatic juice formed in response to various meals may vary in concentration, but not in the relative amounts of the digestive enzymes. According to Babkin (1950), variations in the principal pancreatic enzymes take place in a parallel manner so that a rise or fall in any one enzyme is accompanied by a coincident change in each of the others. His conclusions may have been invalid since the work that supported parallelism was confined to acute experiments.

Guth *et al.* (1956) were unable to demonstrate adaptation due to the lack of sufficient stimulation with the test meal. A fistulated dog was maintained on a standard diet and fed a test meal only on the day of collection. In contrast, rats fed a high carbohydrate diet or a high protein diet for a period of 21 days adapted to the predominant constituent of the diet (Grossman *et al.* 1943a). There was a pronounced increase in amylase on a high carbohydrate diet. A high protein diet resulted in a greatly increased trypsin content. Gidez (1973) found the level of lipase to increase in the rat pancreas when the fat content of the diet was raised from about 5% to 15-22%. Little or no additional increase in lipase levels was obtained by any further increase in the amount of dietary fat. The study by Gidez (1973) showed that the response to changes in diet to be relatively slow. The minimum time period for rats fed a given diet may be 6-10 days before an altered level of

enzymes is elicited. Similarly, Corring (1975) reported that adaptation to the diet in the growing pig requires 5 to 6 days. The mechanism for adaptation is unknown.

Total collections of pancreatic juice and hourly measurements of volume, protein content and enzyme activities between meals for different diets were made to quantitate adaptation in the growing pig. A possible mechanism for adaptation is discussed.

C. Materials and Methods

Experiment 1

Three Yorkshire X Lacombe barrows (30 kg average liveweight) were prepared for collection of pancreatic juice with draining and re-entrant cannulas. The procedure involved the construction of a duodenal pouch at the pancreatic duct and end-to-end anastomosis of the duodenum (Chapter I). Following surgery, the pigs were housed individually in 0.5 x 1.0 m stainless steel metabolism crates and allowed a 2-wk recuperation period. During this time, they had free access to an 18% crude protein starter diet (Table I.1). Water was supplied *ad libitum* from a low pressure drinking nipple.

The three barrows (35 kg average initial weight) were used in a randomized complete block design and fed three experimental diets (Table I.2) twice daily at 0800h and 2000h, 900 g each meal, for a 14-day period for each diet.

The diets were formulated to meet or exceed National Academy of Sciences-National Research Council (NAS-NRC 1979) recommended levels of nutrient requirements, with the exception of protein in the protein-free diet. Dextrose was included at a level of 10% to potentially improve palatability. Analyses for protein ($N \times 6.25$), dry matter, ash, ether extract and energy content were carried out according to the Association of Analytical Chemists (AOAC 1975) methods. The average liveweight of the pigs during this experiment was 42 kg.

Experiment 2

The control and protein-free treatments were repeated with 3 barrows (35 kg average initial weight) fitted with pancreatic cannulas and housed individually in metabolism crates. A randomized complete block design was used. Pigs were fed twice daily at 0800h and 2000h, 900 g each meal, for a 14-day period for each diet. The average liveweight of the pigs during this experiment was 38 kg.

Collection of pancreatic juice

Pancreatic juice was collected on days 9 (0800h to 2000h) and 13 (2000h to 0800h) of each test period for both experiments. The volume of pancreatic juice collected for each hour was measured and 10 ml were subsampled and stored at 5 °C for analysis of protein content and activities of four digestive enzymes (trypsin, chymotrypsin, amylase and

lipase). Pancreatic juice was reintroduced by gravity flow after subsampling at the end of each hourly collection.

Analytical procedures

Analyses were initiated immediately after the completion of each 12 h collection and transport of samples to the laboratory. Protein was determined by the method of Lowry *et al.* (1951) using bovine serum albumin (BSA) as a standard. The activities of trypsin, chymotrypsin, amylase and lipase were measured according to methods described by Bergmeyer (1974). A Gilford® 2600 recording spectrophotometer was used to measure the extinction changes. Chemicals were purchased from Sigma Chemical Co., St. Louis, Missouri.

Trypsin and chymotrypsin. Pancreatic juice was diluted 10-fold with a buffer solution (5 °C) and the proteolytic enzymes were activated with an equal amount of a saturated solution of enterokinase (enteropeptidase; EC 3.4.21.9). The buffer solution (Glazer and Steer 1977) contained 100 µg/ml of BSA, 50 mM CaCl₂, and 50 mM Tris-HCl. The enterokinase solution was made by dissolving 1 g enterokinase in 100 ml distilled and deionized water (5 °C), centrifuging the solution at 16,300g for 20 minutes at 5 °C and decanting the supernatant for immediate use. The enterokinase (5 units/mg) was isolated from porcine intestine as a partially purified salt-free lyophilized powder containing approximately 65% protein (Sigma Chemical Co., St. Louis, Missouri). Mixtures

were incubated for 5 d at 5 °C for trypsinogen activation and for 3 h at 5 °C for chymotrypsinogen activation. These incubation periods were found to be optimal under these and similar conditions described by Gorrill and Thomas (1967) using bovine pancreatic juice. Trypsin activity was measured at 25 °C and pH 8.1 using α -N-toluene-*p*-sulphonyl-L-arginine methyl ester (TAME) as substrate. Chymotrypsin activity was measured at 25 °C and pH 7.8 using N-benzoyl-L-tyrosine ethyl ester (BTEE) as substrate.

Amylase. Pancreatic juice was diluted 200-fold with saline (5 °C). Amylase activity was measured at 25 °C and pH 6.9 using a solution of soluble potato starch (improved Lintner method) as substrate.

Lipase. Pancreatic juice was diluted 2500-fold with distilled and deionized water (5 °C). Lipase activity was measured at 30 °C and pH 8.5 using a gum arabic stabilized emulsion of triolein (olive oil suspension) as substrate.

Statistical analysis

One unit of enzyme activity was defined as the hydrolysis of 1 μ mol substrate in 1 min under optimal conditions. Enzyme activities were reported for each hour of collection as units of activity/ml $\times$ ml pancreatic juice secreted/h. The total values during 12 h collection are the sum of hourly measurements. Analysis of variance procedures were used to detect significant differences in the data and means were compared by Student-Neuman-Keuls' multiple range

test (Steel and Torrie 1980).

D. Results

In both experiments, a trend ($P < 0.05$) for increased volume, protein and all four enzyme activities after feeding was observed (Tables II.1-3, II.5 and II.6). Following an initial increase in volume, protein and enzyme activity within the first 3-4 h after feeding, all parameters decreased to basal levels within 4 h prior to the next feeding. The only exception to this trend was the activity of lipase from pigs fed the high fat diet (Table II.1). In this case, lipase did not show the same rapid increase in activity after feeding but increased within the first 6-7 h following introduction of the meal. This latent period of induction may have been due to the retention of fat in the stomach and its passage into the duodenum at a later time.

In Experiment 1 (Table II.4), dietary treatment had no effect ($P > 0.05$) on daily volume or protein content. Lipase activity increased 6-fold ($P < 0.05$) with the feeding of 10% tallow as compared to 2% tallow included in the diets. Trypsin, chymotrypsin and amylase activities decreased 2-fold ($P < 0.05$) with the absence of dietary protein. In Experiment 2 (Table II.7), dietary treatment had no effect ($P > 0.05$) on daily volume. All four enzyme activities and protein content decreased with the absence of dietary protein ($P < 0.05$).

In both experiments, no significant differences ($P > 0.05$) were found between collections made during the day or night (Tables II.8 and II.9).

E. Discussion

The success with draining pancreatic secretions through a duodenal pouch has provided an alternative to cannulation of the pancreatic duct in the growing pig. The use of a duodenal pouch prevented damage to pancreatic tissue and irritation to the pancreatic duct that could occur with direct duct cannulation. The flow of secretions was continuous from the pouch and the contribution to pancreatic secretions by the pouch has been shown to be less than 0.5% (Chapter I).

It was thought that enterokinase from the mucosa of the duodenal pouch would activate trypsinogen to trypsin. In other preparations, the pouch was flushed with saline and 2 ml of Trasylol® (proteinase inactivator, 10,000 KIU/ml, FBA Pharmaceuticals Ltd., Pointe Claire, Quebec), a trypsin inhibitor, at the end of surgery into the duodenal pouch to prevent autodigestion. The use of Trasylol® was found to be unnecessary, since no measureable amount of active trypsin was found in the secretions collected with or without the use of the inhibitor. The practice of flushing the pouch with saline to remove bile and free enterokinase prevented autodigestion without the use of inhibition.

Enterokinase has been described as a brush border enzyme bound to the surface of the villous epithelial cells of the duodenum and released by cell desquamation (Nordström and Dahlqvist 1970). Hadorn *et al.* (1971) found that free enterokinase activity increased 10-fold with the addition of bile salts to the supernatant of brush border suspensions from the rat intestine. Barns and Elmslie (1977) contend that porcine enterokinase is released or solubilized by the combined action of bile salts and proteolytic enzymes. Porcine enterokinase is readily solubilized by sodium deoxycholate, but not by trypsin or chymotrypsin (Louvard *et al.* 1973). The solubilization of enterokinase by a detergent may be necessary to enable the enzyme to activate trypsin and begin proteolytic digestion. This explains the lack of active trypsin in secretions collected from the duodenal pouch and confers the importance of the one-way valve in preventing backflow of digesta and bile into the pouch.

The luminal contents of the pouch have been washed out by flushing it with saline. This has removed bile and free enterokinase. Although Dragstedt *et al.* (1930) collected pancreatic juice containing active trypsin from a duodenal pouch in the dog, they isolated a larger proportion of duodenum, did not allow the return of pancreatic juice to the remaining duodenum and did not wash out the pouch. Their preparation utilized a single fistula. Perhaps the remaining bile and enterokinase were sufficient to activate trypsinogen within the time of collection.

Traces of an insoluble white precipitate have been found in the secretions collected in this study. Upon microscopic examination of the precipitate in slide smears, uniform particles (1-2 μ diameter) stained purple with Leishman stain were observed. No white blood cells were located, but bacteria were present. Evidence of desquamated intestinal or pancreatic cells along with insoluble mucin were noted in the remnant mosaic patterns found in the slide smears. The particles have not yet been identified.

Enzyme activities were expressed as international units over a given time period (1 h). This expression of total activity allows for summation to obtain between-meal or chronological measurements thereby making it possible to quantitate pancreatic enzyme activities. Volume, protein content and total enzyme activity can also be expressed on a daily basis, in this study, since no differences were found between collections made during the day or night (Tables II.8 and II.9). The expression of volume, protein content and enzyme activity over a given time period can demonstrate not just the quality of pancreatic secretions, but the quantity as well.

The pancreatic enzyme activities measured here on adaptation are comparable to those reported by Corring and Saucier (1972) and Corring (1975). These workers collected pancreatic juice from growing pigs using direct duct cannulation. They showed that when the level of dietary proteins, starch and lipids increased or decreased, the

specific activity of proteolytic enzymes, amylase and lipase increased or decreased in a direct relationship. Currently, adaptation was demonstrated using a duodenal pouch and expressing total activity of pancreatic enzymes in response to a meal on an hourly basis (Tables II.1-II.3, II.5 and II.6).

Corring and Saucier (1972) found a 2.5-fold increase in chymotrypsin activity when pigs were fed a diet with 0% protein and 81% starch adapted to a diet with 30% protein and 41.5% starch. Daily feed intake was approximately 2.0 kg. Trypsin activity increased by 33%. Amylase activity increased by 56%. In Experiment 1 and 2 (Tables II.4 and II.7), pigs fed the control diet (14% protein, 46% starch) as compared to the protein-free diet (0% protein, 79% starch) showed a 2-fold and 3-fold increase in chymotrypsin activity. Trypsin activity increased 2-fold in Experiment 1 and 3.5-fold in Experiment 2. Amylase activity increased by 88% in Experiment 1 and 156% in Experiment 2.

Corring (1975) reported a 7-fold increase in lipase activity and a 25% decrease in amylase activity when pigs that were fed a diet containing 3% oil and 60% starch were fed a diet containing 21% oil and 20% starch. Feed intake was approximately 0.9 kg. Similarly in Experiment 1 (Table II.4), pigs fed the high fat diet (10% tallow, 38% starch) as compared to the control diet (2% tallow, 46% starch) showed a 6-fold increase in lipase activity, but no significant difference ($P > 0.05$) in amylase activity.

With the exception of amylase activity, values reported for enzyme activities fall within the range reported in the literature for growing pigs (Corring 1980, Zebrowska *et al.* 1981, Partridge *et al.* 1982). Although the increase in total activity of the other enzymes could be attributed to an increase in feed intake and the subsequent increase in secretion, the lower amylase activity could not be explained.

The control of pancreatic secretion has been divided into three phases: a cephalic phase under nervous control, a gastric phase under nervous and hormonal control, and an intestinal phase under hormonal control. Pavlov (1910) proved the existence of a psychic influence on the secretion rate by the pancreas in dogs. Stimulation of the thoracic vagus nerves in pigs under anaesthesia caused a profuse flow of pancreatic juice containing high concentrations of amylase and bicarbonate (Hickson 1963). Preshaw *et al.* (1965) have shown that gastrin and hydrochloric acid (HCl), released by acetylcholine from the pyloric gland area, stimulates the protein and bicarbonate output by the pancreas. Secretin isolated from duodenal mucosa responds to stomach acid and causes the release of water and electrolytes, but has relatively little effect on the secretion of enzymes and proteins (Wang and Grossman 1951). These results suggest a mechanism for the cephalic phase. A neural impulse caused by sensory or conditioned stimuli travels along the vagus nerve and causes gastrin, HCl and

secretin to be released which stimulates pancreatic secretion. In the present study, pigs were conditioned to meal-feeding. The increase in volume, protein content and enzyme activities in Experiment 1 (Tables II.1-II.3) and Experiment 2 (Tables II.5 and II.6) immediately after introducing the meal may be due to a cephalic phase.

A vago-vagal reflex has been proposed as the mechanism for the gastric phase of pancreatic secretion. Gastric distension has been shown to increase volume and protein output from the pancreas of dog and man (White *et al.* 1960). The action was blocked by vagotomy, atropine or procaine infiltration of the vagi in the neck. In addition, extracts of antral mucosa have produced a humorally mediated increase in pancreatic enzyme secretion (Blair *et al.* 1961).

In studies of the intestinal phase of pancreatic secretion, Harper and Raper (1943) isolated a hormone, pancreozymin, from the duodenal mucosa that stimulated the production of pancreatic enzymes. They determined that pancreozymin acted directly upon the cells of the pancreas to stimulate enzyme production in a parallel manner. Occasionally their preparations of pancreozymin showed very slight secretin activity, but the majority had no effect on the rate of flow of the juice.

The release of pancreozymin from the duodenal mucosa and the increase in the secretion of pancreatic enzymes is dependent on the presence of digestion products (Wang and Grossman 1951). In studies using dogs with chronic gastric

and pancreatic fistulas, the perfusion of a variety of fat and protein digestion products into the small intestine have been shown to stimulate pancreatic secretion (Meyer and Jones 1974, Meyer *et al.* 1976a, Meyer *et al.* 1976b, Meyer and Kelly 1976). The perfusion of amino acids, glucose or fatty acids into the duodenum of 45 people caused an increase in pancreatic enzyme output (Go *et al.* 1970). In particular, the enzyme output due to the essential amino acids was significantly higher ($P < 0.05$) than that evoked by the fatty acids, glucose or nonessential amino acids. Similarly, pigs fed the protein-free diet decreased pancreatic enzyme output according to adaptation in Experiment 1 (Table II.4) and parallelism in Experiment 2 (Table II.7). Pigs in Experiment 2 were of a lighter weight (38 kg) than pigs in Experiment 1 (46 kg) and may have been more sensitive to the lack of dietary protein. As a result, pigs in Experiment 2 reduced the loss of endogenous protein by depressing all four enzyme activities. The absence of protein or amino acids in the diet appears to have a significant effect on pancreatic secretions in a parallel manner. In contrast, dietary fat had a dramatic effect on lipase activity in an adaptive fashion (Table II.4).

Grossman *et al.* (1943b) hypothesized that the hydrolyzed form of a feedstuff may determine the enzyme composition of pancreatic secretion and that this may occur by a hormonal or humoral mechanism. Ben Abdeljlil and Desnuelle (1964) showed that levels of pancreatic amylase in

rats fed a diet containing 75% glucose were similar to those in rats fed a diet containing 75% starch. They concluded that the product of hydrolysis, glucose, and the resulting elevation of plasma glucose concentrations brought about increased amylase biosynthesis. Lavau *et al.* (1974) provided evidence for a humoral mechanism for lipase and amylase induction by subjecting rats to intravenous glucose, lipid and amino acids. Lipase activity increased with the infusion of lipid and amylase activity increased with the infusion of glucose. Since infused amino acids did not affect pancreatic enzyme composition, they concluded that protease induction may require the release of duodenal hormones rather than an increase of the blood amino acid pool.

Corring (1977) has suggested that for each intestinal pool of hydrolysis products: amino acids, glucose or fatty acids, there is a corresponding duodenal factor acting specifically on the biosynthesis of proteolytic enzymes, amylase or lipase. Morrisset and Dunnigan (1967) have shown that nervous control is not involved in adaptation and proposed that pancreozymin may exist in different forms specific for the stimulation of proteolytic enzymes, amylase or lipase synthesis and release. Since their proposal, additional polypeptides acting on pancreatic secretion have been found in the intestinal mucosa. Vasoactive intestinal polypeptide stimulates electrolyte and water secretion with an apparent efficiency of 5-10% that of secretin (Said and Mutt 1972). Adelson and Ehrlich (1972) have reported two

factors from porcine duodenal mucosa which release amylase from pancreatic zymogen granules *in vitro*. Adelson and Rothman (1974) isolated a peptide, chymodenin, from porcine duodenum which, on injection into rats, caused a specific release in chymotrypsinogen secretion by the pancreas. Duodenal factors are also suggested in the adaptation of amylase and trypsinogen to the diet by Dick and Felber (1975).

In the present study, the adaptive capability of the pancreas appeared to be dependent on the inclusion of protein in the diet. Parallelism was followed by adaptation once a requirement for protein was met. The lack of adaptation by amylase may be due to a saturation effect as demonstrated by Gidez (1973).

Adaptation is a means to reduce endogenous protein loss and possibly to optimize total digestibilities by matching enzyme activity to substrate. The saturation effect could control the rate of hydrolysis and ensure that absorption kinetics were optimal for all hydrolysis products. If the rate of hydrolysis was a limiting factor, adaptation could also increase energy stores when substrates are in excess of maintenance and growth requirements by ensuring their breakdown for absorption.

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TABLE II.1. Hourly volume, protein and enzyme activities during 12 hour collection of pancreatic juice from pigs fed the high fat diet (Experiment 1).

HOURS	VOLUME (ml)	PROTEIN (g)	TRYPsin'	CHYMOTRYPSIN'	AMYLASE'	LIPASE'
0-1	163±67	1.07±0.31	21.7±4.7	12.0±4.0	4.47±1.78	96±22
1-2	227±155	1.20±0.66	22.0±2.6	11.0±7.0	4.00±0.80	172±100
2-3	250±130	1.07±0.65	15.0±3.5	10.0±2.6	2.50±0.53	71±60
3-4	207±106	0.90±0.56	13.7±5.7	6.3±2.3	2.10±1.15	87±46
4-5	233±80	0.83±0.41	9.3±4.5	8.0±2.6	2.23±0.35	195±53
5-6	227±64	0.83±0.41	7.7±4.0	5.7±2.5	1.97±1.06	429±326
6-7	210±26	0.63±0.35	6.3±3.5	5.0±2.6	1.27±0.57	305±350
7-8	117±31	0.27±0.12	5.0±2.0	2.7±1.2	0.47±0.35	187±138
8-9	83±31	0.23±0.12	1.7±0.6	2.0±1.0	0.23±0.23	131±85
9-10	93±50	0.27±0.15	1.7±0.6	3.7±2.5	0.47±0.31	216±162
10-11	63±29	0.13±0.06	1.3±0.6	2.0±0.0	0.77±0.72	29±23
11-12	87±40	0.23±0.06	3.3±1.5	3.0±2.6	0.77±0.72	57±28

' Enzyme activities are expressed as u/h x 1000.

' Mean ± standard deviation; 6 observations per mean.

TABLE II.2. Hourly volume, protein and enzyme activities during 12 hour collection of pancreatic juice from pigs fed the control diet (Experiment 1).

HOURS	VOLUME (ml)	PROTEIN (g)	TRYPsin'	CHYMOTRYPsin'	AMYLase'	LIPase'
0-1	118±48'	1.02±0.41	12.2±6.7	7.8±2.3	2.70±1.08	39±33
1-2	150±73	0.88±0.42	18.2±8.3	8.8±6.9	3.33±1.66	30±15
2-3	228±64	1.08±0.42	17.8±6.3	12.8±4.0	2.60±0.61	57±19
3-4	223±71	0.92±0.34	11.8±3.9	11.2±4.4	1.90±0.80	38±8
4-5	178±135	0.83±0.41	13.8±6.6	7.2±3.9	1.72±0.99	29±11
5-6	172±106	0.63±0.33	11.0±1.3	6.0±2.6	1.48±1.42	22±11
6-7	218±84	0.57±0.27	6.5±3.3	7.0±2.6	1.22±0.30	33±11
7-8	200±79	0.47±0.31	9.0±5.7	6.2±2.8	0.70±0.28	19±8
8-9	160±89	0.42±0.30	7.0±2.3	3.8±2.9	0.42±0.17	20±10
9-10	110±58	0.22±0.15	6.2±4.0	2.8±1.5	0.32±0.16	11±5
10-11	148±71	0.30±0.18	5.2±3.3	4.0±2.1	0.30±0.11	19±13
11-12	90±55	0.20±0.06	3.0±1.7	5.5±5.1	0.33±0.32	10±5

' Enzyme activities are expressed as u/h x 1000.

' Mean ± standard deviation; 6 observations per mean.

TABLE II.3. Hourly volume, protein and enzyme activities during 12 hour collection of pancreatic juice from pigs fed the protein-free diet (Experiment 1).

HOURS	VOLUME (ml)	PROTEIN (g)	TRYPSIN ¹	CHYMOTRYPSIN ¹	AMYLASE ¹	LIPASE ¹
0-1	177±48	0.93±0.31	8.0±4.4	5.2±1.8	1.38±0.80	30±18
1-2	155±36	0.78±0.30	9.7±5.4	4.3±1.0	1.17±0.72	37±30
2-3	198±43	0.83±0.25	6.3±3.7	4.3±1.0	0.98±0.24	37±26
3-4	233±107	0.90±0.25	6.7±3.0	4.7±2.8	1.25±0.52	39±32
4-5	215±72	0.68±0.13	5.7±4.1	3.8±1.0	0.68±0.28	19±15
5-6	172±73	0.50±0.06	4.2±2.3	3.0±0.6	0.67±0.31	35±23
6-7	157±90	0.50±0.21	4.2±2.5	3.0±1.1	0.75±0.59	18±16
7-8	138±77	0.45±0.14	3.2±1.8	2.5±1.0	0.58±0.39	25±32
8-9	78±56	0.23±0.08	1.7±0.5	1.5±0.5	0.47±0.37	19±18
9-10	90±46	0.30±0.11	3.2±0.8	2.0±0.6	0.50±0.33	17±8
10-11	57±21	0.20±0.09	2.0±1.1	1.3±0.5	0.32±0.24	7±3
11-12	68±16	0.25±0.10	3.0±0.6	1.3±0.5	0.28±0.25	6±4

¹ Enzyme activities are expressed as u/h x 1000.

² Mean ± standard deviation; 6 observations per mean.

TABLE II.4. Total volume, protein and enzyme activities during 12 hour collection of pancreatic juice from pigs fed the high fat, control and protein-free diets (Experiment 1).

DIET	VOLUME (ml)	PROTEIN (g)	TRYPSIN ¹	CHYMOTRYPSIN ¹	AMYLASE ¹	LIPASE ¹
High Fat	1960a ²	7.66a	108.7b	71.2b	21.25b	1975b
Control	1995a	7.54a	121.7b	83.1b	17.02b	327a
Protein Free	1738a	6.55a	57.9a	36.9a	9.03a	289a
SE ³	82	0.39	7.3	5.8	1.60	28

¹ Enzyme activities are expressed as u/12 h x 1000.

² a-b, means in a given column with the same letter are not significantly different ($P < 0.05$).

³ SE, standard error of the mean; 6 observations per mean.

TABLE II.5. Hourly volume, protein and enzyme activities during 12 hour collection of pancreatic juice from pigs fed the control diet (Experiment 2).

HOURS	VOLUME (ml)	PROTEIN (g)	TRYPsin'	CHYMOTRYPsin'	AMYLASE'	LIPASE'
0-1	160±36'	0.85±0.30	18.8±8.2	9.8±2.1	2.57±0.99	20±11
1-2	108±41	0.70±0.37	12.2±3.9	7.0±3.5	1.88±0.61	11±8
2-3	117±62	0.55±0.24	13.0±4.5	6.2±3.2	1.02±0.26	25±23
3-4	145±52	0.58±0.16	13.7±4.2	8.7±2.7	1.12±0.40	17±12
4-5	107±36	0.48±0.21	11.3±2.9	4.8±2.1	0.72±0.32	10±7
5-6	143±62	0.57±0.19	12.2±2.1	6.5±3.6	0.73±0.26	24±30
6-7	177±64	0.57±0.18	9.0±3.5	7.3±4.2	1.23±0.61	21±8
7-8	123±45	0.38±0.16	10.2±3.4	5.3±3.4	0.53±0.21	14±14
8-9	120±64	0.40±0.13	6.8±2.1	6.0±4.4	0.70±0.52	29±43
9-10	127±80	0.28±0.12	6.2±3.0	8.2±5.5	0.62±0.34	15±17
10-11	60±47	0.18±0.12	3.7±2.9	2.5±1.9	0.35±0.38	5±4
11-12	52±31	0.20±0.09	3.5±2.3	3.2±2.8	0.42±0.21	12±13

' Enzyme activities are expressed as u/h x 1000.

' Mean ± standard deviation; 6 observations per mean.

TABLE 11.6. Hourly volume, protein and enzyme activities during 12 hour collection of pancreatic juice from pigs fed the protein-free (Experiment 2).

HOURS	VOLUME (ml)	PROTEIN (g)	TRYPSIN ¹	CHYMOTRYPSIN ¹	AMYLASE ¹	LIPASE ¹
0-1	127±58	0.43±0.23	4.5±2.2	3.5±2.5	0.85±0.52	7±4
1-2	100±28	0.33±0.10	4.3±1.5	3.0±1.7	0.65±0.39	4±3
2-3	102±13	0.37±0.14	3.5±0.8	2.8±1.9	0.58±0.31	7±6
3-4	128±17	0.30±0.06	3.8±1.6	2.5±1.2	0.48±0.15	12±7
4-5	171±62	0.38±0.08	4.3±1.2	2.5±1.8	0.63±0.26	22±20
5-6	85±32	0.18±0.08	1.5±0.5	1.5±0.5	0.20±0.06	11±11
6-7	114±37	0.23±0.08	2.5±1.4	2.2±1.6	0.32±0.18	12±12
7-8	110±46	0.20±0.13	2.7±0.8	2.0±1.5	0.28±0.20	9±5
8-9	86±31	0.15±0.12	1.7±0.8	1.2±0.4	0.15±0.12	6±6
9-10	77±32	0.17±0.08	1.5±0.5	1.7±0.8	0.23±0.28	7±6
10-11	77±51	0.17±0.08	1.8±1.2	1.7±0.8	0.15±0.08	8±4
11-12	60±21	0.15±0.05	1.8±0.4	1.3±0.5	0.12±0.04	7±4

¹ Enzyme activities are expressed as u/h x 1000.

² Mean ± standard deviation; 6 observations per mean.

TABLE II.7. Total volume, protein and enzyme activities during 12 hour collection of pancreatic juice from pigs fed the control and protein-free diets (Experiment 2).

DIET	VOLUME (ml)	PROTEIN (g)	TRYPSIN ¹	CHYMOTRYPSIN ¹	AMYLASE ¹	LIPASE ¹
Control	1439a ²	5.74b	120.6b	75.5b	11.89b	203b
Protein Free	1237a	3.06a	33.9a	25.9a	4.64a	112a
SE ³	128	0.32	3.6	3.2	0.60	22

¹ Enzyme activities are expressed as u/12 h x 1000.

² a-b, means in a given column with the same letter are not significantly different ($P < 0.05$).

³ SE, standard error of the mean; 6 observations per mean.

TABLE II.8. Total volume, protein and enzyme activities during day and night collection of pancreatic juice from pigs fed the high fat, control and protein-free diets (Experiment 1).

TREATMENT		VOLUME (ml)	PROTEIN (g)	TRYPSIN ¹	CHYMOTRYPSIN ¹	AMYLASE ¹	LIPASE ¹
High Fat	D ^a	1997a ¹	8.04a	110.2a	73.8a	23.20a	2028b
	N	1923a	7.28a	107.2a	68.6a	19.30a	1922b
Control	D	2126a	7.33a	119.1a	82.0a	16.93a	281a
	N	1864a	7.74a	124.3a	84.2a	17.11a	373a
Protein Free	D	1680a	5.76a	50.3a	35.7a	8.26a	259a
	N	1796a	7.34a	65.0a	38.1a	9.80a	319a
SE ¹		116	0.55	10.3	8.2	2.26	40

¹ Enzyme activities are expressed as u/12 h x 1000.

¹ D=day (0800h to 2000h), N=night (2000h to 0800h).

¹ a-b, means in a given column with the same letter are not significantly different ($p < 0.05$).

¹ SE, standard error of the mean; 3 observations per mean.

TABLE II.9. Total volume, protein and enzyme activities during day and night collection of pancreatic juice from pigs fed the control and protein-free diets (Experiment 2).

TREATMENT		VOLUME (ml)	PROTEIN (g)	TRYPSIN ¹	CHYMOTRYPSIN ¹	AMYLASE ¹	LIPASE ¹
Control	D ¹	1341a ¹	6.55a	120.7b	68.0b	11.80a	194a
	N	1537a	4.93a	120.3b	83.0b	11.98a	212a
Protein Free D	D	1371a	3.20a	31.3a	31.7a	4.78a	127a
	N	1104a	2.92a	36.5a	20.1a	4.50a	97a
SE ⁴		181	0.45	5.1	4.5	0.85	31

¹ Enzyme activities are expressed as u/12 h x 1000.

² D=day (0800h to 2000h), N=night (2000h to 0800h).

³ a-b, means in a given column with the same letter are not significantly different ($P < 0.05$).

⁴ SE, standard error of the mean; 3 observations per mean.

III. EFFECT OF FEEDING ON PANCREATIC SECRETIONS

A. Abstract

The influence of feeding on enzyme secretion by the pancreas was determined in growing pigs. Four barrows (35 kg average initial weight) were fitted with pancreatic cannulas and used in a randomized complete block design. The four pigs (46 kg average liveweight) were fed a 16% crude protein grower diet in 1 meal (1.8 kg at 0800h), in 3 meals (0.6 kg at 0800h, 1600h and 2400h) and in 2 meals (0.9 kg at 0800h and 2000h) for a period of 2 weeks for each treatment. Pancreatic juice was collected during 24 h on days 9 and 13 of each test period. Hourly volume, protein and activities of four enzymes (trypsin, chymotrypsin, amylase and lipase) were measured. Volume, protein and all four enzyme activities usually increased ($P < 0.05$) after feeding. Daily volume increased ($P < 0.01$) by 0.5 l with each additional meal. Frequency of feeding had no effect ($P > 0.01$) on protein secretion and proteolytic enzyme activities. Amylase activity increased 2-fold ($P < 0.01$) with each additional meal. Lipase activity was higher ($P < 0.01$) when pigs were fed 1 meal per day as compared to 2 meals per day. Feeding stimulated pancreatic secretion and frequency of feeding increased daily volume and amylase activity.

Key Words: CANNULATION, FEEDING FREQUENCY, PANCREATIC ENZYMES, SWINE

B. Introduction

The secretion of pancreatic juice is continuous in the growing pig (Kvasnitskii 1951, Pekas 1965, Corring *et al.* 1972, Partridge *et al.* 1982). Corring *et al.* (1972) reported that the consumption of a meal increased both volume and protein output with a maximum being reached 2 to 3 h after feeding. In contrast, Partridge *et al.* (1982) were unable to demonstrate an increase in pancreatic secretion after feeding. In further studies, Low (1982) found that the pattern of hourly activity of trypsin and chymotrypsin measured in duodenal contents indicated a response to feeding and demonstrated continuous activity during 24 h.

In the present study, total daily collections of pancreatic juice and hourly measurements of volume, protein content and enzyme activities were made to quantitate pancreatic secretion by the growing pig in response to feeding and frequency of feeding.

C. Materials and Methods

Four Yorkshire X Lacombe barrows (30 kg average liveweight) were prepared for collection of pancreatic juice with draining and re-entrant cannulas. The procedure involved the construction of a duodenal pouch at the pancreatic duct and end-to-end anastomosis of the duodenum (Chapter I). Following surgery, the pigs were individually housed in 0.5 x 1.0 m stainless steel metabolism crates and allowed a 2-wk recuperation period. During this time, they

had free access to a 16% crude protein grower diet (Table III.1). Water was supplied *ad libitum* from a low pressure drinking nipple.

The four barrows (35 kg average initial weight) were used in a randomized complete block design. This design was used to avoid cephalic stimulation of pancreatic secretion since experimental animals were fed and housed in the same barn. Pigs were fed a grower diet in 1 meal (1.8 kg at 0800h), in 3 meals (0.6 kg at 0800h, 1600h and 2400h) and in 2 meals (0.9 kg at 0800h and 2000h) for a period of 2 weeks for each treatment. The grower diet was formulated to meet or exceed National Academy of Sciences-National Research Council (NAS-NRC 1979) recommended levels of nutrient requirements. Analyses for protein ($N \times 6.25$), dry matter, ash, ether extract and energy content were carried out according to the Association of Analytical Chemists (AOAC 1975) methods. The average liveweight of the pigs during this trial was 46 kg.

To measure basal levels of secretion, two pigs that had been fed 2 meals per day were starved for 36 h on days 4 and 8 following the experiment and pancreatic juice was collected during the last 24 h of this 36 h fast.

Collection of pancreatic juice

Pancreatic juice was collected during 24 h on days 9 and 13 of each experimental period and on days 4 and 8 following the experiment. The volume of pancreatic juice was

measured each hour and 10 ml were subsampled and stored at 5 °C for analysis of protein content and activities of four digestive enzymes (trypsin, chymotrypsin, amylase and lipase). Pancreatic juice was reintroduced by gravity flow after subsampling at the end of each hourly collection.

Analytical procedures

Analyses were initiated immediately following the completion of each 24 h collection and transport of samples to the laboratory. Protein was determined by the method of Lowry *et al.* (1951) using bovine serum albumin (BSA) as a standard. Enzyme assays were conducted as reported in Chapter II. A Gilford® 2600 recording spectrophotometer was used to measure the extinction changes. Chemicals were purchased from Sigma Chemical Co., St. Louis, Missouri.

Statistical analysis

One unit of enzyme activity was defined as the hydrolysis of 1 μ mol substrate in 1 min under optimal conditions. Enzyme activities were reported for each hour of collection as units of activity/ml x ml pancreatic juice secreted/h. The total values for daily, between-meal, post-prandial and pre-prandial collection are the sum of hourly measurements. Analysis of variance procedures were used to detect significant differences in the data and means were compared by Student-Neuman-Keuls' multiple range test (Steel and Torrie 1980).

D. Results

The effect of feeding frequency on hourly volume, protein and enzyme activities are shown in Figures III.1-6 and Tables III.2-4. With once-a-day feeding, the rate of secretion remained relatively stable during the 24 h collection whereas protein and the level of activity of all four enzymes increased with the introduction of the meal followed by a decrease to basal levels. Characteristic repeating patterns were obtained when the animals were fed 2 or 3 times each day. Volume, protein and enzyme activity showed a steady decline to basal levels until the next feeding time.

In general, post-prandial measurements were found to be greater ($P < 0.05$) than pre-prandial measurements (Tables III.5-7). Protein content and amylase activity increased ($P < 0.05$) after the meal when pigs were fed 1 meal per day whereas volume, trypsin, chymotrypsin and lipase activity did not show this trend (Table III.5). In contrast when pigs were fed 2 or 3 meals per day, volume, protein and all four enzymes increased ($P < 0.05$) after the meal except for lipase activity from pigs fed twice-a-day (Tables III.6 and III.7). It is of interest to note that lipase activity increased just prior to the last feeding of the day for pigs fed 3 meals per day (Figure III.6). No significant differences ($P > 0.05$) were found in between-meal measurements (Tables III.8 and III.9). Daily volume tended to increase ($P < 0.01$) by 0.5 l with each additional meal

(Table III.10). Frequency of feeding had no effect ($P > 0.01$) on protein secretion and proteolytic enzyme activities. Amylase activity increased 2-fold ($P < 0.01$) with each additional meal. Lipase activity was higher ($P < 0.01$) when pigs were fed 1 meal per day as compared to 2 meals per day.

To measure the basal levels of secretion, two pigs that had been fed 2 meals per day were starved for 36 h on days 4 and 8 following the experiment (Table III.11). Pancreatic secretions increased to 88% of the volume that one would have expected if animals had been fed. Concurrently, protein content increased to 81%, trypsin activity increased to 70%, chymotrypsin activity increased to 86%, amylase activity increased to 61% and lipase activity increased to 72% of expected norms. Following this initial increase at 12-13 h after the last meal, volume, protein and all four enzyme activities rapidly declined to basal levels as shown by the low level of average hourly secretion for the following 12 h (Table III.11). Pancreatic secretions did not increase ($P < 0.05$) at the following expected meal-time and remained at basal levels.

E. Discussion

The control of pancreatic secretion is divided into cephalic, gastric and intestinal phases. The major part of the response by the pancreas during digestion is dependent on the passage of acid and digestion products of protein and

fat along the small intestine (Harper 1972).

It is of interest to note that when pigs were intentionally not fed at their regular meal time pancreatic secretions increased in anticipation of feeding and quickly decreased to basal levels until the next feeding. By conditioning the pigs to meal-feeding, it appears that the cephalic phase may be primarily responsible for the rapid increase in the secretion of pancreatic juice immediately after feeding and that the gastric phase is masked by the effects of the cephalic phase. The intestinal phase may be seen in the gradual decrease in pancreatic secretions after feeding and appears to be responsible for initiating the synthesis and release of pancreatic enzymes as seen in adaptation to the diet.

Pigs fed 3 meals per day secreted more amylase whereas pigs fed 1 meal per day secreted more lipase when compared to pigs fed 2 meals per day. It appears that pigs fed 2 meals per day may have secreted a more balanced enzyme complement.

Many scientists have investigated the influence of frequency of feeding on weight gain and feed conversion efficiency in pigs. Pshenichny (1958) compared feeding twice, 3 times and 4 times daily and found that feeding sows twice daily results in more efficient use of the feed. Barber *et al.* (1961) found no significant differences between pigs fed once or twice per day. Cromwell *et al.* (1965) reported that pigs fed two times daily gained

significantly faster than pigs fed once daily by 7%.

Passback, Jr. *et al.* (1968) found that pigs fed twice per day were more efficient than pigs fed once per day by 14%. In contrast, growth rate and feed efficiency were better in pigs fed once a day than in those fed twice a day by 4 and 3% respectively (Van Kempen *et al.* 1979). Lu and Ma (1981) showed that total weight gain and feed conversion efficiency in pigs fed twice daily were higher than for pigs fed once or three times daily. Although controlled feeding systems are more labor intensive, pigs tested present better performance fed two times a day when compared to *ad libitum* feeding (Ludwig *et al.* 1981). The costs per kg gain were found to be lower for pigs fed two meals per day when compared to pigs fed one or three meals per day (Caleffi and Broccaioli 1981).

Feeding pigs two meals per day may be optimal for performance. The continuous secretion of pancreatic juice by pigs fed 1 meal per day and excessive stimulation of pancreatic secretion from pigs fed 3 meals per day may be wasteful. As Snook (1968) suggested, a large meal may cause digestive tract loading, whereas *ad libitum* feeding would lead to more frequent pancreatic stimulation. The rate of food passage should also be taken into consideration since it increases with the frequency of feeding (Cromwell *et al.* 1965) and may be important in the rate of hydrolysis and absorption kinetics. Cunningham (1971) reported that the peak absorption of nutrients from the digestive tract of the

pig occurs 3 to 6 h after feeding. These factors could contribute to better assimilation of the diet and feeding 2 meals per day may be best.

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TABLE III.1, Formulation and partial chemical composition of the grower diet.

Ingredients(% as fed):

Wheat	31.5
Barley	50.0
Soybean meal	15.0
Calcium phosphate	1.0
Calcium carbonate	1.0
Iodized salt	0.5
Vitamin-mineral premix'	1.0

Chemical analyses:

Dry Matter(%)	89.0
Gross Energy(MJ/kg)	15.9
Ether Extract(%)	1.4
Crude Protein(%)	16.8
Ash(%)	5.3

The premix provided the following per kg of diet:

(1) minerals - 120 mg zinc, 48 mg manganese, 100 mg iron, 10 mg copper, .1 mg selenium;

(2) vitamins - 7500 IU vitamin A, 700 IU vitamin D₃, 45 IU vitamin E, 12 mg riboflavin, 40 mg niacin, 25 mg pantothenic acid, 28 µg B₁₂.

TABLE III.2. Hourly volume, protein and enzyme activities during 24 hour collection of pancreatic juice from pigs fed one meal per day.

HOURS	VOLUME (ml)	PROTEIN (g)	TRYPSIN ¹	CHYMOTRYPSIN ¹	AMYLASE ¹	LIPASE ¹
0-1	204±99	1.18±0.53	12.1±9.2	12.8±6.2	2.83±2.06	22±7
1-2	187±89	0.95±0.70	8.8±5.8	10.8±5.0	2.04±1.60	23±12
2-3	256±95	1.07±0.62	7.5±2.7	13.6±4.3	1.55±0.83	21±7
3-4	224±62	0.82±0.35	7.1±2.2	11.3±4.1	1.55±1.60	28±9
4-5	181±41	0.70±0.24	5.3±1.8	8.3±2.2	0.96±0.70	19±4
5-6	263±99	0.90±0.40	7.0±2.1	12.0±5.0	1.30±0.80	23±7
6-7	256±71	0.79±0.25	7.8±2.3	10.5±3.8	1.12±0.85	31±11
7-8	256±60	0.74±0.20	6.9±2.6	9.9±3.7	0.72±0.46	20±6
8-9	284±68	0.77±0.23	7.3±1.5	9.8±3.5	1.00±0.61	16±8
9-10	283±57	0.64±0.14	6.4±1.6	10.3±3.9	1.00±0.71	36±11
10-11	215±95	0.55±0.19	4.9±2.0	8.3±4.7	0.74±0.51	36±16
11-12	241±70	0.66±0.27	5.1±1.2	8.8±4.7	0.75±0.44	26±11
12-13	271±66	0.64±0.22	6.4±1.5	9.1±3.2	0.64±0.46	17±5
13-14	243±73	0.56±0.21	6.0±2.6	9.0±3.9	0.56±0.50	23±9
14-15	273±83	0.71±0.37	5.8±2.4	10.4±3.7	0.91±0.38	21±7
15-16	330±88	0.95±0.46	9.1±2.5	11.5±3.3	0.81±0.63	27±11
16-17	254±80	0.67±0.28	6.0±2.2	9.0±3.4	0.71±0.45	22±10
17-18	376±42	0.81±0.18	8.6±2.0	14.1±2.8	0.94±0.51	42±9
18-19	263±23	0.70±0.15	6.8±1.9	9.4±1.6	0.95±0.68	25±10
19-20	196±53	0.52±0.22	5.8±2.4	7.1±2.4	0.61±0.43	11±3
20-21	298±102	0.75±0.42	9.6±3.7	10.0±3.0	0.52±0.31	25±10
21-22	141±72	0.36±0.25	4.0±2.5	5.3±2.6	0.29±0.12	9±2
22-23	229±77	0.56±0.19	6.0±4.3	9.1±4.7	0.40±0.42	23±12
23-24	191±64	0.37±0.23	4.5±2.1	6.3±3.2	0.20±0.12	23±9

¹ Enzyme activities are expressed as u/h x 1000.

² Mean ± standard deviation; 8 observations per mean.

³ Pigs were fed 1.8 kg diet at 0800h (0 HOURS).

TABLE III.3. Hourly volume, protein and enzyme activities during 24 hour collection of pancreatic juice from pigs fed two meals per day.

HOURS	VOLUME (ml)	PROTEIN (g)	TRYPSIN ¹	CHYMOTRYPSIN ¹	AMYLASE ¹	LIPASE ¹
0-1 ¹	349±54 ¹	0.91±0.20	14.8±2.6	17.4±2.4	2.96±0.87	23±8
1-2	330±56	0.79±0.22	11.4±2.5	14.8±4.4	2.34±0.84	7±3
2-3	330±64	0.62±0.13	8.8±3.5	12.8±5.0	2.06±1.15	12±4
3-4	274±64	0.60±0.22	7.5±2.8	12.8±4.6	1.62±0.61	8±4
4-5	263±60	0.54±0.17	6.4±3.0	11.0±2.7	1.60±0.80	17±7
5-6	243±62	0.51±0.25	5.4±2.8	9.0±2.6	1.35±0.68	16±4
6-7	256±74	0.47±0.21	6.9±2.5	9.6±4.1	1.42±0.85	23±7
7-8	229±37	0.44±0.11	5.8±2.4	8.6±3.5	1.15±0.51	7±3
8-9	243±108	0.45±0.17	5.1±2.3	9.5±3.1	1.10±0.73	10±5
9-10	205±64	0.40±0.09	3.8±1.6	7.3±4.1	0.75±0.40	8±3
10-11	235±95	0.32±0.15	4.6±1.9	6.9±4.4	1.01±0.58	10±5
11-12	160±68	0.27±0.10	3.9±1.5	7.3±4.4	0.44±0.34	3±1
12-13	285±95	0.81±0.31	13.1±3.2	13.5±3.9	2.60±1.28	16±10
13-14	298±37	0.81±0.31	9.4±2.4	12.8±3.1	2.35±0.92	9±3
14-15	324±35	0.65±0.20	8.8±2.3	13.0±4.6	2.42±1.08	10±4
15-16	324±84	0.74±0.21	9.1±1.5	11.8±4.6	2.39±1.10	14±9
16-17	320±67	0.64±0.21	9.3±3.2	12.0±4.6	2.21±0.96	10±3
17-18	314±36	0.56±0.18	8.9±3.1	11.9±3.1	1.94±0.76	11±3
18-19	303±65	0.57±0.30	6.3±2.9	10.4±3.6	1.75±1.00	17±7
19-20	331±111	0.57±0.34	7.1±2.9	11.3±4.4	1.71±1.03	14±4
20-21	211±42	0.44±0.18	4.9±2.0	5.8±1.8	1.05±0.61	20±8
21-22	271±81	0.64±0.34	6.3±3.5	8.1±3.2	1.12±1.04	12±4
22-23	198±71	0.34±0.11	4.9±1.8	5.1±2.0	0.51±0.44	9±5
23-24	258±70	0.34±0.15	5.3±1.9	6.3±3.0	0.61±0.36	4±3

¹ Enzyme activities are expressed as u/h x 1000.

¹ Mean ± standard deviation; 8 observations per mean.

¹ Pigs were fed 0.9 kg diet at 0800h and 2000h (0 and 12 HOURS).

TABLE III.4. Hourly volume, protein and enzyme activities during 24 hour collection of pancreatic juice from pigs fed three meals per day.

HOURS	VOLUME (ml)	PROTEIN (g)	TRYPsin ¹	CHYMOTRYPsin ¹	AMYLASE ¹	LIPASE ¹
0-1 ¹	374±59 ¹	1.29±0.89	11.4±3.5	18.3±4.6	6.50±2.40	43±12
1-2	365±62	0.91±0.27	8.0±1.9	15.1±2.6	4.51±2.73	21±11
2-3	325±47	0.74±0.23	6.4±2.7	11.8±2.6	3.67±1.08	19±11
3-4	246±70	0.60±0.30	4.4±1.7	9.3±2.3	2.99±1.56	14±12
4-5	214±57	0.54±0.26	3.8±1.5	8.6±3.1	2.74±1.08	9±8
5-6	273±59	0.71±0.25	5.6±1.6	9.8±3.2	3.76±1.39	13±11
6-7	236±93	0.50±0.23	3.3±2.1	7.4±2.1	3.49±1.87	5±2
7-8	214±45	0.52±0.27	3.5±1.9	6.6±1.8	2.27±0.50	6±2
8-9 ¹	381±62	1.19±0.50	8.8±2.0	16.1±2.9	4.39±2.70	28±14
9-10	346±54	0.797±0.57	5.0±1.7	12.9±5.3	4.47±2.06	15±7
10-11	278±71	0.56±0.21	3.8±1.5	9.1±3.1	3.51±2.41	10±7
11-12	253±80	0.61±0.30	5.1±2.8	8.6±3.6	2.42±1.67	13±7
12-13	275±104	0.47±0.15	5.6±2.3	12.4±7.5	2.64±1.87	8±3
13-14	279±67	0.52±0.26	4.8±1.9	9.0±3.0	3.10±1.69	12±5
14-15	238±81	0.45±0.35	3.6±1.8	8.6±3.2	2.29±1.29	10±4
15-16	268±88	0.47±0.24	4.3±1.6	10.1±5.2	2.57±1.81	26±11
16-17 ¹	341±41	0.81±0.12	10.4±3.8	14.0±2.6	5.29±2.16	14±7
17-18	348±61	0.76±0.15	7.6±5.0	14.0±3.9	4.96±2.33	5±2
18-19	414±67	0.82±0.28	7.4±6.4	16.5±2.6	5.64±1.84	9±3
19-20	339±98	0.62±0.21	4.8±2.3	13.3±3.5	4.29±1.59	16±4
20-21	283±37	0.56±0.21	4.8±2.1	10.8±3.1	2.52±1.09	14±6
21-22	283±37	0.50±0.16	6.3±5.0	9.9±3.4	1.96±1.41	16±5
22-23	221±61	0.36±0.22	3.5±1.7	8.0±2.9	1.41±0.61	12±4
23-24	273±89	0.50±0.30	4.0±2.1	9.0±1.9	1.07±0.63	12±5

¹ Enzyme activities are expressed as u/h x 1000.

² Mean ± standard deviation; 8 observations per mean.

³ Pigs were fed 0.6 kg diet at 0800h, 1600h and 2400h (0, 8 and 16 HOURS).

TABLE III.8. Post-prandial and pre-prandial measurements of volume, protein and enzyme activities during 24 hour collection of pancreatic juice from pigs fed one meal per day.

HOURS	VOLUME (ml)	PROTEIN (g)	TRYPSIN ¹	CHYMOTRYPSIN ¹	AMYLASE ¹	LIPASE ¹
0-12 ¹	2849a ¹	9.77b	86.2a	126.4a	15.56b	301a
12-24	3064a	7.50a	78.3a	110.3a	7.54a	268a
SE ¹	268	1.08	8.8	10.7	2.64	44

¹ Enzyme activities are expressed as u/12 h x 1000.

¹ a-b, means in a given column with the same letter are not significantly different ($P < 0.05$).

¹ Pigs were fed 1.8 kg diet at 0800h (0 HOURS).

¹ SE, standard error of the mean; 8 observations per mean.

TABLE III.6. Post-prandial and pre-prandial measurements of volume, protein and enzyme activities during 24 hour collection of pancreatic juice from pigs fed two meals per day.

HOURS	VOLUME (ml)	PROTEIN (g)	TRYPSIN ¹	CHYMOTRYPSIN ¹	AMYLASE ¹	LIPASE ¹
0-6 ¹	1788b ¹	3.97b	54.3b	77.8b	11.93b	84a
6-12	1328a	2.35a	30.1a	49.2a	5.87a	62a
12-18 ¹	1858b	4.21b	59.6b	75.0b	13.91b	70a
18-24	1572ab	2.90a	34.8a	47.0a	6.75a	76a
SE ¹	112	0.33	4.9	5.5	1.18	16

¹ Enzyme activities are expressed as U/6 h x 1000.

¹ a-b, means in a given column with the same letter are not significantly different ($P < 0.05$).

¹ Pigs were fed 0.9 kg diet at 0800h and 2000h (0 and 12 HOURS).

¹ SE, standard error of the mean; 8 observations per mean.

TABLE III.7. Post-prandial and pre-prandial measurements of volume, protein and enzyme activities during 24 hour collection of pancreatic juice from pigs fed three meals per day.

HOURS	VOLUME (ml)	PROTEIN (g)	TRYPSIN ¹	CHYMOTRYPSIN ¹	AMYLASE ¹	LIPASE ¹
0-4 ¹	1310ab ¹	3.54a	30.2b	54.5bc	17.67cd	97b
4-8	937a	2.27a	16.2a	32.4a	12.26abc	33a
8-12 ¹	1258ab	3.43a	22.7ab	46.7abc	14.79bcd	66ab
12-16	1060a	1.91a	18.3ab	40.1ab	10.60ab	56ab
16-20 ¹	1442b	3.01a	30.2b	57.8c	20.18d	44ab
20-24	1060a	1.92a	18.6ab	37.7ab	6.96a	54ab
SE ¹	77	0.39	3.3	3.9	1.56	12

¹ Enzyme activities are expressed as u/4 h X 1000.

a-d, means in a given column with the same letter are not significantly different (P<0.05).

¹ Pigs were fed 0.6 kg at 0800h, 1600h and 2400h (0, 8 and 16 HOURS).

¹ SE, standard error of the mean; 8 observations per mean.

TABLE III.8. Between-meal measurements of volume, protein and enzyme activities during 24 hour collection of pancreatic juice from pigs fed two meals per day.

HOURS	VOLUME (ml)	PROTEIN (g)	TRYPSIN ¹	CHYMOTRYPSIN ¹	AMYLASE ¹	LIPASE ¹
0-12 ¹	3117a ¹	6.32a	84.4a	127.0a	17.80a	146a
12-24 ¹	3437a	7.11a	94.4a	122.0a	20.66a	146a
SE ²	173	0.55	8.7	9.9	2.21	44

¹ Enzyme activities are expressed as u/12 h x 1000.

² a, means in a given column with the same letter are not significantly different ($P < 0.05$).

³ Pigs were fed 0.9 kg diet at 0800h and 2000h (0 and 12 HOURS).

⁴ SE, standard error of the mean; 8 observations per mean.

TABLE III.9. Between-meal measurements of volume, protein and enzyme activities during 24 hour collection of pancreatic juice from pigs fed three meals per day.

HOURS	VOLUME (ml)	PROTEIN (g)	TRYPSIN ¹	CHYMOTRYPSIN ¹	AMYLASE ¹	LIPASE ¹
0-8 ¹	2247 ^a	5.81 ^a	46.4 ^a	87.9 ^a	29.93 ^a	130 ^a
8-16 ¹	2318 ^a	5.34 ^a	41.0 ^a	86.8 ^a	25.39 ^a	122 ^a
16-24 ¹	2502 ^a	4.93 ^a	48.8 ^a	95.5 ^a	27.14 ^a	98 ^a
SE ²	191	0.89	7.5	9.9	4.34	27

¹ Enzyme activities are expressed as u/8 h X 1000.

² a, means in a given column with the same letter are not significantly different ($P < 0.05$).

³ Pigs were fed 0.6 kg diet at 0800h, 1600h and 2400h (0, 8 and 16 HOURS).

⁴ SE, standard error of the mean; 8 observations per mean.

TABLE III. 10. Total volume of pancreatic juice and enzyme activities during 24 hour collection of pancreatic juice from pigs fed one, two or three meals per day.

TREATMENT	VOLUME (ml)	PROTEIN (g)	TRYPSIN ¹	CHYMOTRYPSIN ¹	AMYLASE ¹	LIPASE ¹
One meal	5913a ¹	17.37a	164.5a	236.7a	23.10a	569b
Two meals	6554ab	13.43a	178.8a	249.0a	38.46b	292a
Three meals	7067b	16.08a	136.2a	269.2a	82.46c	350ab
SE ¹	180	0.89	7.4	10.1	6.66	45

¹ Enzyme activities are expressed as u/24 h x 1000.

¹ a, means in a given column with the same letter are not significantly different ($P < 0.01$).

¹ SE, standard error of the mean; 8 observations per mean.

TABLE III.11. Average hourly measurements of volume, protein and enzyme activities during 24 hour collection of pancreatic juice from pigs during a 36 hour fast.

HOUR	VOLUME (ml)	PROTEIN (g)	TRYPSIN ¹	CHYMOTRYPSIN ¹	AMYLASE ¹	LIPASE ¹
12-13	278±65 ²	0.70±0.14	9.8±2.2	13.3±6.2	1.70±0.60	14±9
12-24	86±77 ²	0.36±0.28	4.9±3.2	4.1±3.4	0.98±0.61	4±3
24-25	82±62 ²	0.10±0.05	3.0±1.7	4.0±3.0	0.70±0.31	3±2
24-36	78±59 ²	0.26±0.23	3.0±2.0	2.8±1.6	0.89±0.64	3±2
35-36	73±45 ²	0.15±0.10	2.3±1.3	3.3±3.2	0.60±0.37	3±2

¹ Enzyme activities are expressed as u/h x 1000.

² Mean ± standard deviation; 4 observations per mean in a given row.

³ Mean ± standard deviation; 48 observations per mean in a given row.

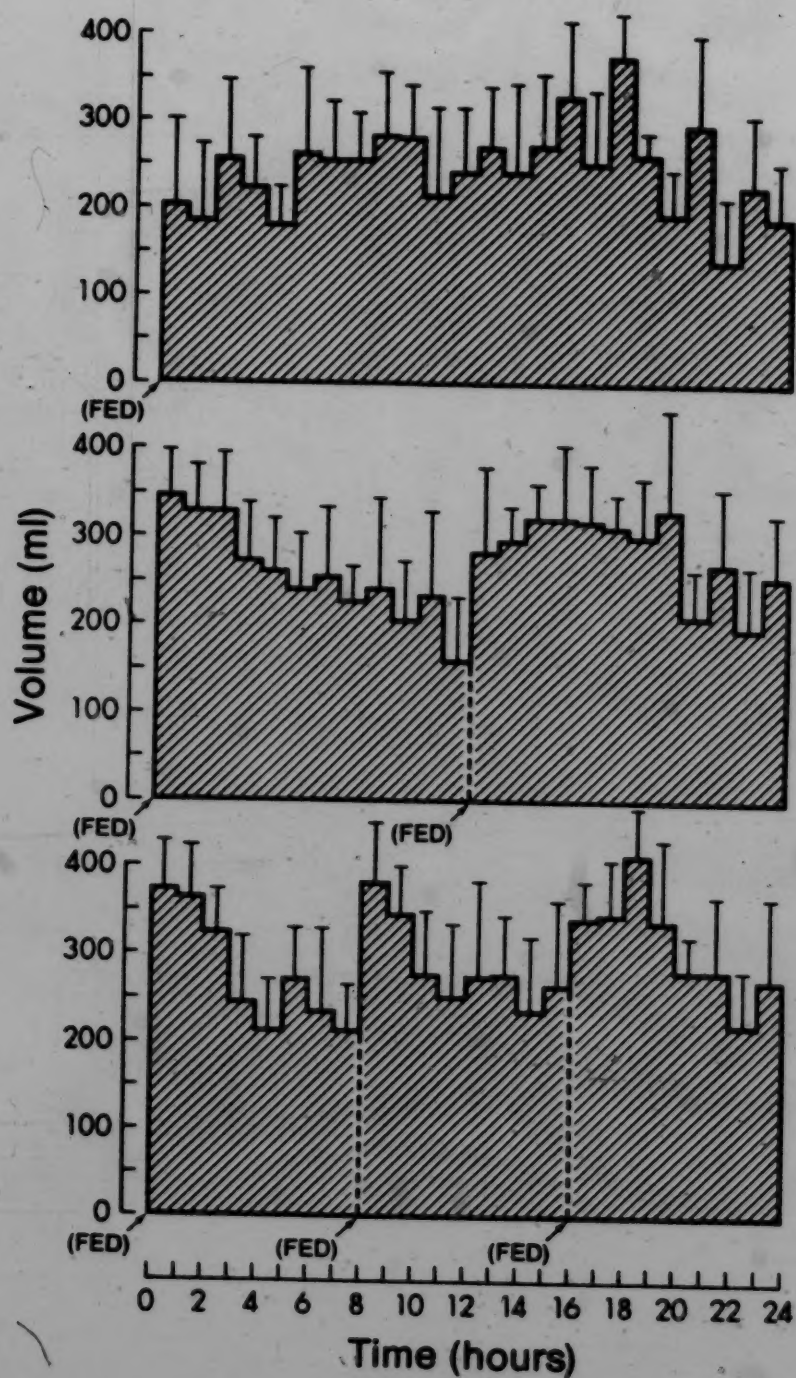


Figure III.1. Hourly volume of pancreatic secretions from feeding one, two or three times a day.

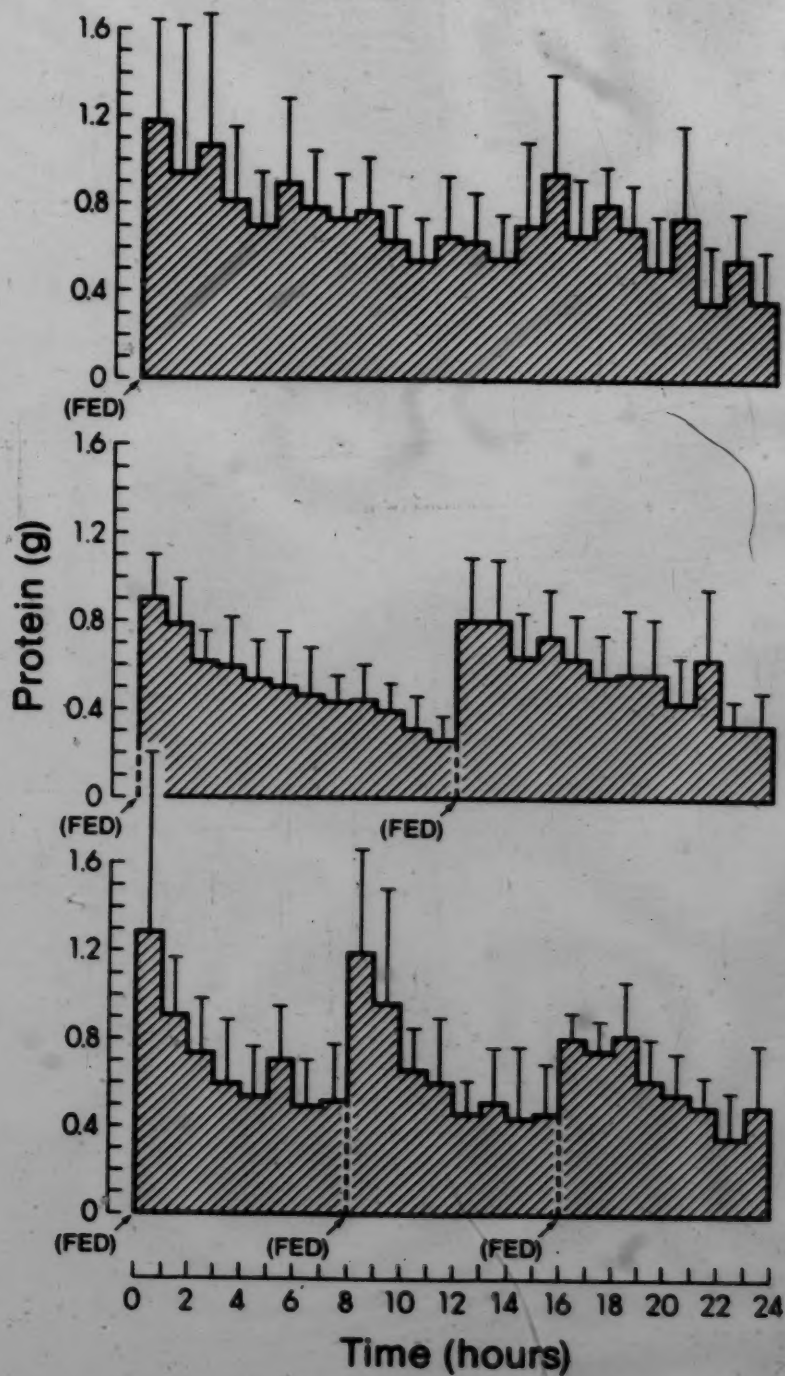


Figure III.2. Protein content of hourly pancreatic secretions from feeding one, two or three times a day.

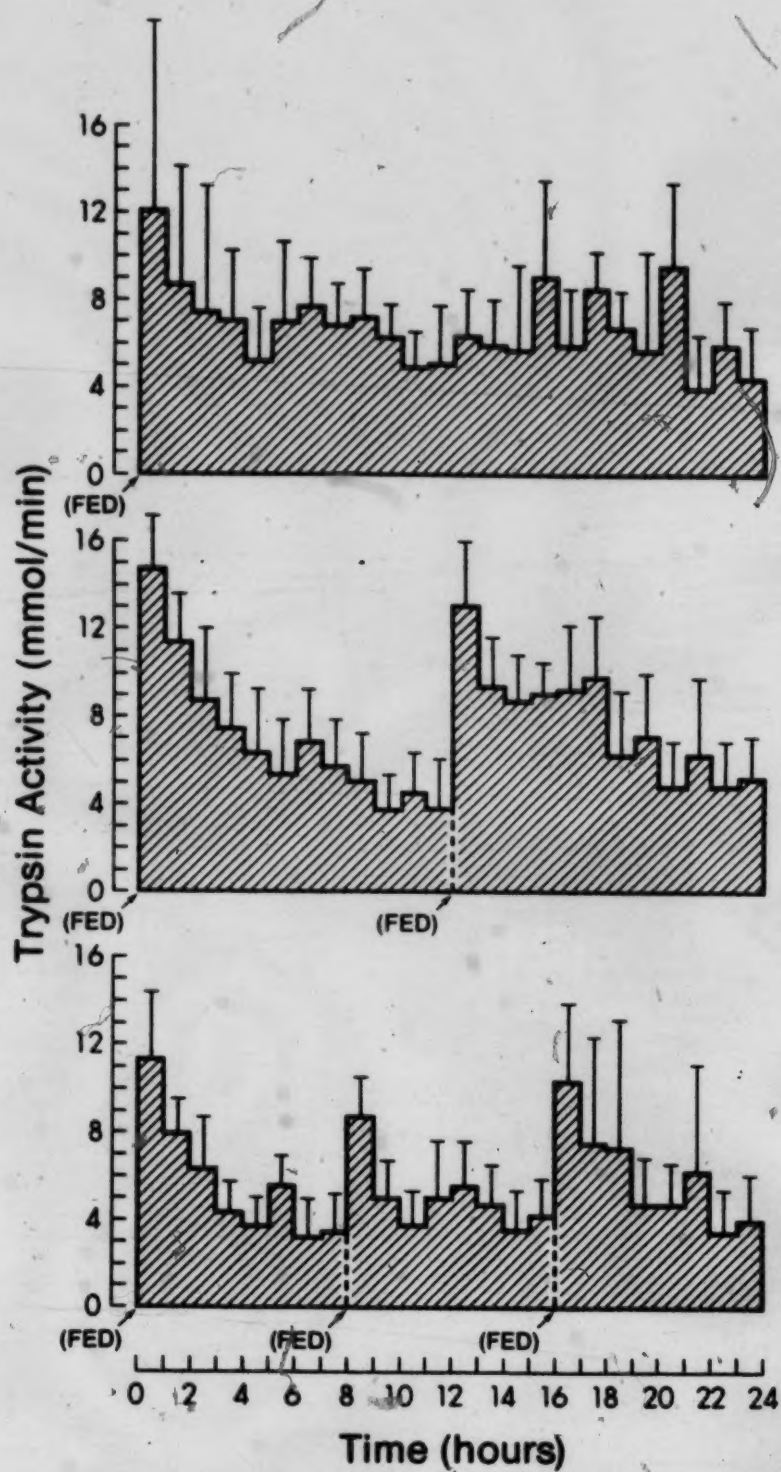


Figure III.3. Trypsin activity of hourly pancreatic secretions from feeding one, two or three times a day.

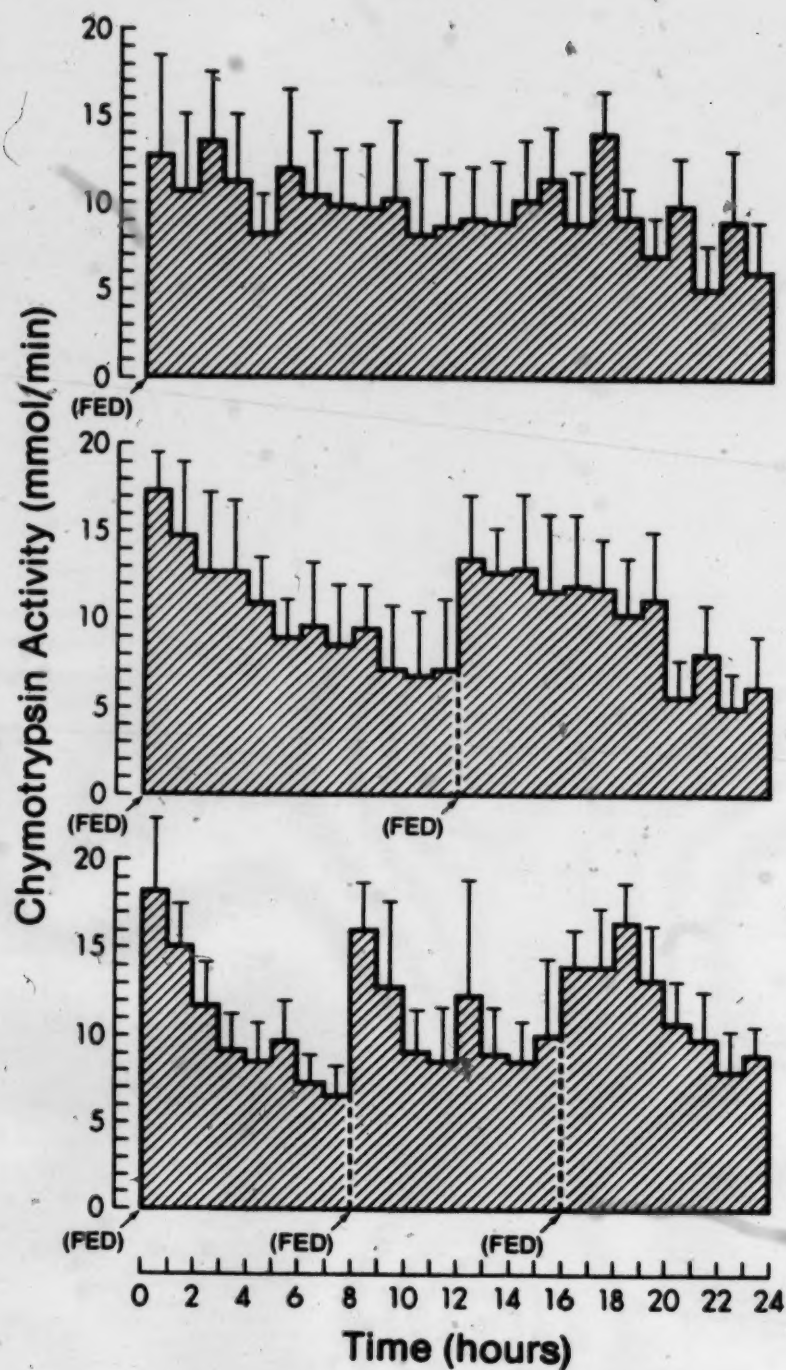


Figure III.4. Chymotrypsin activity of hourly pancreatic secretions from feeding one, two or three times a day.

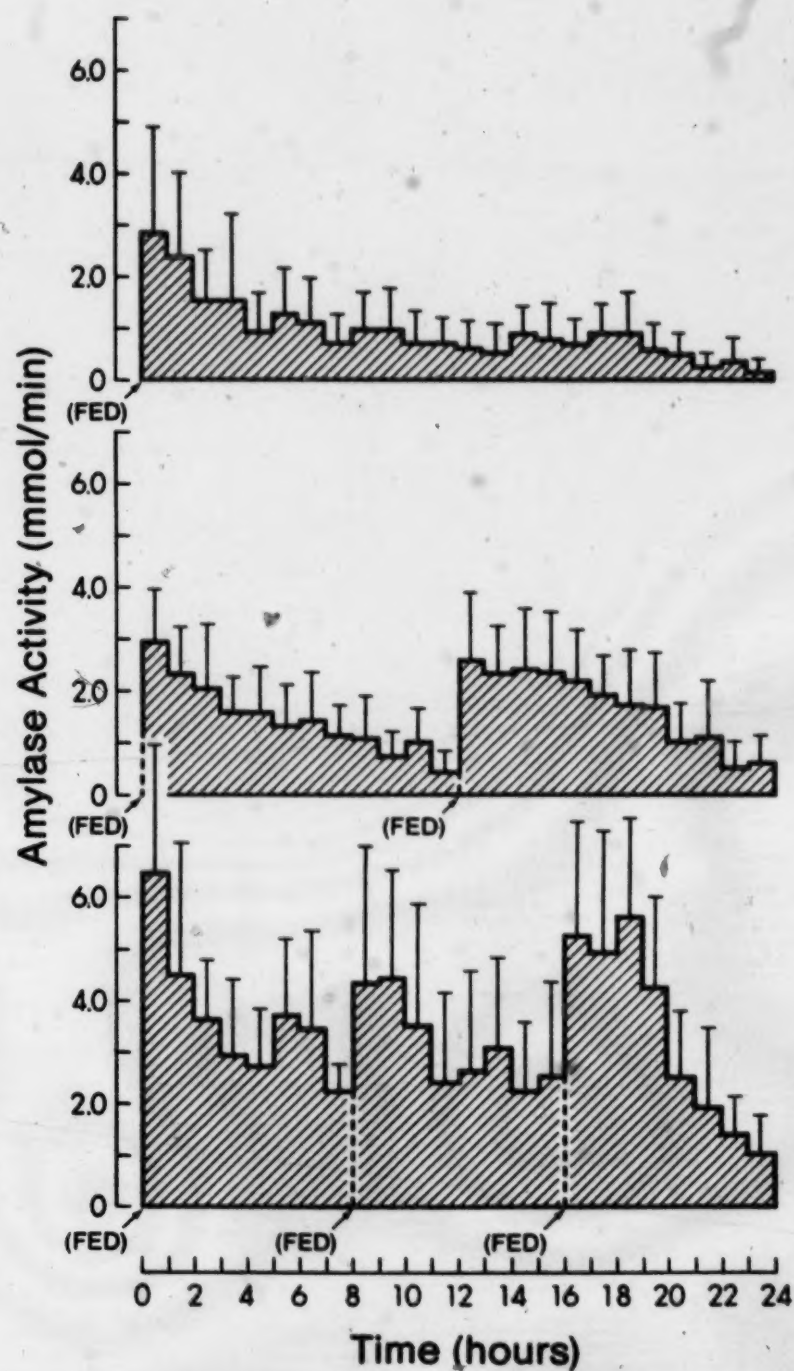


Figure III.5. Amylase activity of hourly pancreatic secretions from feeding one, two or three times a day.

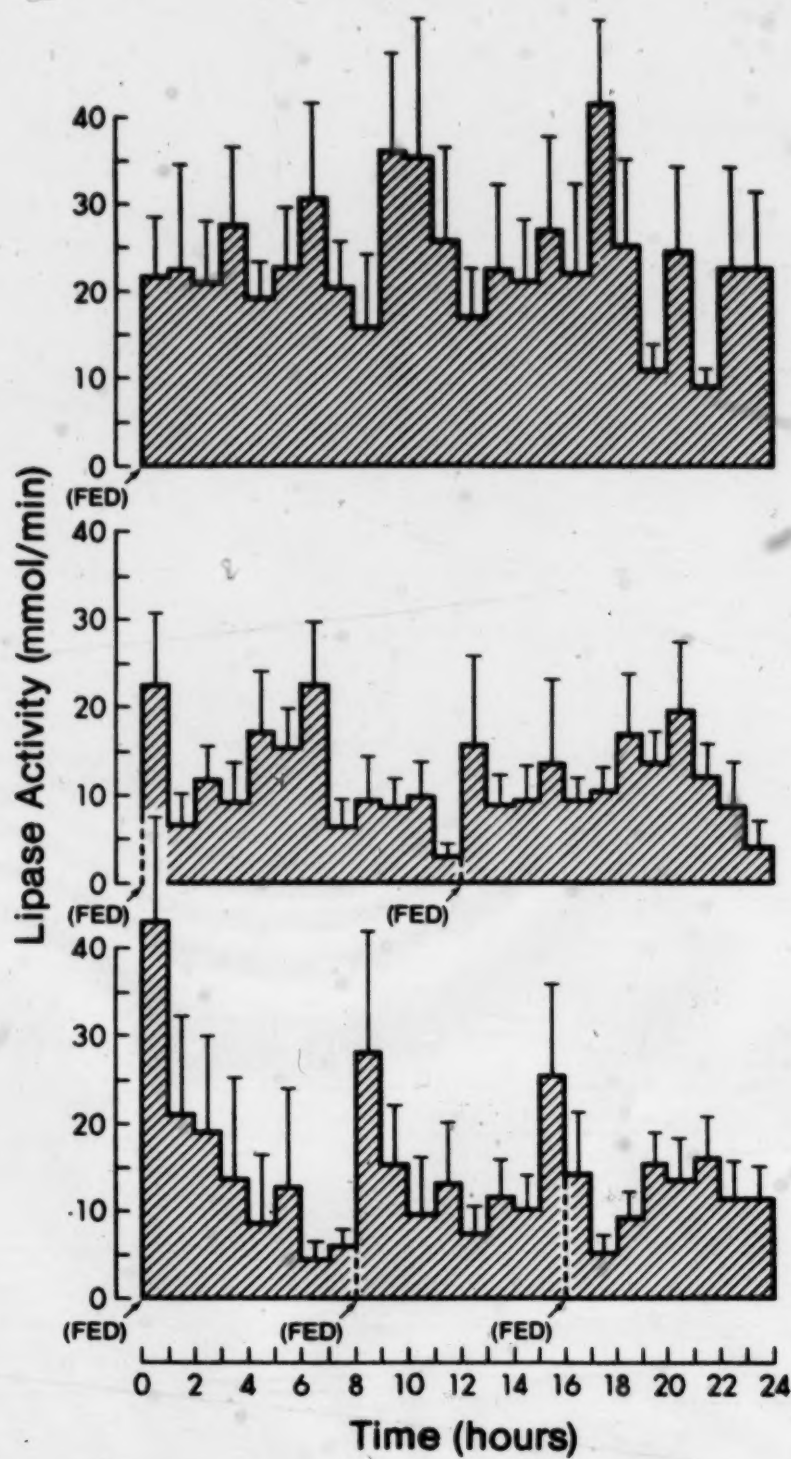


Figure III.6. Lipase activity of hourly pancreatic secretions from feeding one, two or three times a day.

GENERAL SUMMARY AND CONCLUSIONS

The pig is an excellent model for the study of pancreatic secretions. The pancreas of the pig was found to be a distinct, bilobed structure with a single, functional duct that entered the duodenum directly. Total collection of pancreatic juice could be made by the use of direct duct cannulation or the formation of a duodenal pouch at the pancreatic duct and cannulation of the pouch. The latter was chosen in this study to prevent damage to pancreatic tissue and irritation to the pancreatic duct. Cannulation of a duodenal pouch gave permanent access to total pancreatic secretions and was determined to be a promising alternative to direct duct cannulation.

The secretion of pancreatic juice was shown to be continuous, to adapt in response to diet composition and to increase in response to feeding and frequency of feeding. Pancreatic secretions were not interrupted by surgery. Volume, protein content and enzyme activities were consistent from day to day subject to the experimental conditions.

The daily volume and proteolytic enzyme activities of pancreatic juice secreted was dependent on the type of diet fed. Pigs fed a partially-purified control diet in Experiment 1 secreted approximately 4.0 l which contained 15.1 g protein whereas pigs fed a cereal-based diet in Experiment 3 secreted approximately 6.5 l which

contained 15.6 g protein. A reversal in trypsin and chymotrypsin activity was found between the two experiments. Trypsin activity was higher and chymotrypsin activity was lower with pigs fed the partially-purified diets as compared to pigs fed the cereal-based diet. This may be due to the differences in the sources of dietary protein.

The complement of pancreatic enzymes secreted appeared to be dependent on the composition of the diet. Lipase showed a 6-fold ($P < 0.05$) increase in activity with the feeding of 10% tallow as compared to 2% tallow in the diets (Experiment 1). Proteolytic activity decreased 2-fold ($P < 0.05$) with the absence of dietary protein (Experiment 1). The lack of dietary protein had a marked influence ($P < 0.05$) in depressing all four enzyme activities (trypsin, chymotrypsin, amylase and lipase) and protein content in Experiment 2. The pigs in this experiment appeared to have been more sensitive compared to pigs in Experiment 1 to the lack of dietary protein and to have decreased the synthesis and release of digestive enzymes in a parallel fashion thereby reducing the loss of endogenous protein.

Three phases have been described in the literature regarding the control of pancreatic secretion: a cephalic phase under nervous control, a gastric phase under nervous and hormonal control, and an intestinal phase under hormonal control. The intestinal phase to

pancreatic secretion is thought to mediate exocrine pancreatic adaptation to the diet. The products of digestion may initiate the message to the pancreas to alter the complement of enzymes secreted by causing the release of gastrointestinal hormones not yet identified to increase the synthesis and release of specific digestive enzymes. A positive feedback mechanism between the activity of a digestive enzyme (trypsin/chymotrypsin, amylase or lipase) and the corresponding size of the intestinal pool of hydrolysis products (amino acids, glucose or fatty acids) may exist.

The adaptive capability of the pancreas is dependent on the level of protein in the diet. Without adequate dietary protein, the pancreas secretes enzymes in a parallel manner. This reduces the loss of endogenous protein and may help in the animal's nitrogen balance. In contrast, adaptation may be a strategy to increase energy stores when substrates are in excess of maintenance and growth requirements. In this situation, the rate of hydrolysis of protein, carbohydrates and fats may be limiting without adaptation.

The results of the first two experiments suggested that pancreatic secretions follow a characteristic profile in response to feeding. Post-prandial flow rates increased within the first 3 h. Maximum volumes were secreted within 3-7 h and were followed by a decline

during the last 5 h. Protein content and enzyme activities increased immediately after feeding and gradually decreased to basal levels before the next meal. No significant differences ($P > 0.05$) in volume, protein content and enzyme activities were found between 12 h collections made during the day or night when pigs were fed at 0800h and 2000h.

The results from Experiment 3 showed that pancreatic secretions increase in response to feeding. Feeding appeared to stimulate pancreatic secretion. It is of interest to note that when pigs were intentionally not fed at their regular meal time, pancreatic secretions increased in anticipation of feeding and quickly decreased to basal levels until the next feeding. By conditioning the pigs to meal-feeding, it appears that the cephalic phase may be primarily responsible for the rapid increase in the secretion of pancreatic juice immediately after feeding and that the gastric phase is masked by the effects of the cephalic phase. The intestinal phase may be seen in the gradual decrease in pancreatic secretions and appears to be responsible for initiating the synthesis and release of pancreatic enzymes.

The frequency of feeding was shown to increase ($P < 0.01$) daily volume by 0.5 l and amylase activity 2-fold with each additional meal, but to have no effect ($P > 0.01$) on protein secretion and proteolytic enzyme

activities. Numerically, pigs fed 2 meals per day secreted less protein (NS) and appear to have secreted a balanced enzyme complement which may be optimal for the growing pig. The continuous secretion by pigs fed 1 meal per day and excessive stimulation from pigs fed 3 meals per day may be wasteful, but was not shown to be significant. Growth trials and digestibility studies could answer how effective is the stimulation of pancreatic secretions on feed conversion efficiency and digestibilities of feedstuffs at different frequencies and levels of feeding.

8

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